

EFFECTS OF WETTING AND DRYING ON CARBON AND NITROGEN  
MINERALIZATION IN SELECTED COASTAL PLAIN SOILS

by

JOHN SAYLER KRUSE

(Under the direction of David E. Kissel, Ph.D.)

ABSTRACT

Carbon and nitrogen mineralization from soil organic matter are important components of nutrient cycling and crop nutrient availability. The objective of this study was to determine effects of repeated wetting and drying of soils on rates of C and N mineralization. The study compared mineralization rates in three soils, utilizing cotton leaves or compost residues. One set of treatments was subjected to repeated drying and rewetting and the other was kept at constant moisture content. Rates of C mineralization were measured in the treatment containers. Mineralized N was measured by leaching with 0.01 M CaCl<sub>2</sub> periodically, for 185 d. Carbon mineralization rates were not significantly affected by moisture regime. Both residue and soil type affected rates of C mineralization. For N mineralization, moisture effect was not significant in control soils with no residue, but did significantly affect mineralization from residue.

INDEX WORDS: Carbon mineralization, Nitrogen mineralization, Wetting and drying,  
Crop residues

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JOHN SAYLER KRUSE

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JOHN SAYLER KRUSE

Approved:

Major Professor: David Kissel

Committee: Miguel Cabrera  
Paul Hendrix

Electronic Version Approved:

Gordon L. Patel  
Dean of the Graduate School  
The University of Georgia  
July 2002

## **DEDICATION**

This thesis is dedicated to my wife, Lyn, and to my children Johnny, Katie, and Noah Kruse. Your patience and understanding, as well as your prayers, made this possible. God bless you.

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## INTRODUCTION

The spatial variability of soils in crop production fields makes it difficult to predict chemical and biological processes due to variations in soil properties such as organic matter, clay content, water content, and available nutrients. These factors may influence the rates of C and N mineralization to a significant degree.

Despite the difficulty in predicting mineralization, much research has been conducted to develop an accurate predictor of mineralization. There are good reasons, both economic and environmental, to predict C and N mineralization correctly. In a study by Cabrera and Kissel (1988), mineralized N varied from 31 to 51 kg N ha<sup>-1</sup> on a conventionally tilled soil, and from 21 to 107 kg N ha<sup>-1</sup> in a fallow field over 127 days in May through September. This may represent a substantial portion of the nitrogen needs of a crop over a growing season. Better knowledge of actual mineralization rates would allow a producer to forego the expense of purchasing and applying surplus N. Additionally, concern has increased dramatically among the scientific community over human influence on the global N cycle. World N fertilizer use increased from 10 Tg yr<sup>-1</sup> in 1960 to 80 Tg yr<sup>-1</sup> in 1990. A tally of world crops revealed only 53 Tg N were harvested in 1990, indicating only 2/3 as much N was harvested as was applied. This excess N can become a source of groundwater and stream contamination (Frink et al., 1999).

A more thorough understanding of the factors that influence both C and N mineralization should lead to N fertilizer applications that better reflect the actual needs

of the crop. This process would begin with determining the N needs of the crop based on expected yield, calculating the amount of N that will be available from mineralization over time and space, then subtracting mineralized N from the crop N needs to determine the N fertilizer required. The nitrogen available from the soil depends on the initial available soil N, the type and quantity of crop residues returned to the soil, N added from rainfall, as well as N losses from volatilization and leaching (Neeteson, 1990). One obstacle to the aforementioned method is the fact that mineralization is primarily biologically driven. Often temperature and moisture factors cannot be controlled even though they have a profound influence on C and N mineralization rates (Birch, 1958).

Repeated wetting and drying, as occurs in the field, may have a pronounced effect on mineralization rates. As soil water content reaches wilting point microbial activity decreases (Stark and Firestone, 1995). However, when a soil is rewetted microbial activity, as measured by respiration, begins again in a few hours (Lundquist et al., 1999). Rates of mineralization are highest soon after a soil is wetted to field capacity (-0.01 MPa), then taper off over time. Soils with frequent drying and rewetting cycles exhibit a decrease in the magnitude of the flushes over time due to a depletion of the organic reserves (Birch, 1958).

The objective of this research was to quantify the effect of wetting and drying on mineralization of C and N from soil organic matter, and from decomposing organic residues incorporated into the soil. While many previous studies have indicated the importance of soil moisture levels as a factor in C and N mineralization, they often maintained the incubations at constant moisture levels throughout the study. It was hypothesized that the wetting and drying cycles would increase net C and N mineralized

over a control maintained at constant moisture. The concept was to more accurately reflect actual field conditions that are subject to periodic rain episodes interspersed with periods of drying.

Soils selected for this study were obtained from an agricultural production field that is conventionally tilled. The soils differed in clay and organic matter content, since these factors vary widely and may influence mineralization. Cotton leaves were included in the study at a rate that approximately reflects field residue amounts. A compost was also incorporated into the study to provide a contrast to the cotton residue. Many municipalities and companies throughout the United States are composting organic materials from municipal wastes. Compost may become an increasingly available source of organic residue in the future.

## LITERATURE REVIEW

### Introduction

Decomposition by microorganisms is the primary means by which organic carbon and nitrogen are converted to inorganic forms in the soil (Tisdale et al., 1999). Factors that influence the population growth, mobility, nutrient consumption, and respiration of these microorganisms have a direct effect on the rates of C and N mineralization. Studies reveal that the environmental factors: moisture, temperature, pH, oxygen, and soil properties, affect C and N mineralization rates to differing degrees.

### Effects of residues, soil texture, and soil organic matter on N mineralization

Other than N fertilizer, decomposing crop residues and soil organic matter typically represent the single largest source of mineral N that is utilized by a crop. Determining and predicting N mineralization rates based on crop residue quality and quantity has been the subject of many previous studies. In a study of leguminous crop residues in soil, Frankenberger and Abdelgamid (1985) reported that the N mineralized was correlated with the N concentration, and was curvilinear to the C:N ratio. Specifically, the relationship between percentage N mineralized and the C:N ratio of incorporated plant residues best fit the equation:  $Y = bX^m$  where  $b$  represents the intercept and  $m$  the slope. They note this expression shows an inverse curvilinear relationship between the percentage of N mineralized and increasing C:N ratio of the residues with a highly

significant correlation of  $r = 0.88$ . Palm and Sanchez (1991), Oglesby and Fownes (1992), and Tian et al. (1992) used the polyphenolic concentrations in residues to predict mineralized N, whereas Müller et al. (1988), and Kirchmann and Birgqvist (1989) demonstrated that the lignin concentration of the residue was a useful predictor of N mineralization rates. Vigil and Kissel (1991) combined six experiments from the literature with two conducted by the authors to determine general relationships between net N mineralized and the chemical characteristics of the residue. They predicted N mineralized based on the C:N ratio, N concentration, and lignin concentration in the residues. At 50% and 100% field capacity moisture regimes, Das et al. (1993) predicted N mineralization rates on the C:N ratio and N concentration. Fox et al. (1990), Constantinides and Fownes (1994), and De Neve' and Hofman (1996) evaluated combinations of these factors in predicting N mineralization from residues. Fox et al. found a significant correlation ( $r^2 = 0.866$ ) between incorporated legume (lignin + polyphenol):N ratio and N mineralization. Using incorporated vegetable crop residues, De Neve and Hofman reported the amount of mineralized organic N was better correlated to the C:N ratio of residue lignin, than the amount of total N, with 78% of the total variance of mineralized organic N explained. Constantinides and Fownes, however, concluded that differences among previous studies, in which chemical predictors were best, arose from relatively small ranges in chemical composition of materials used. In materials representing a wide range of chemical composition, initial soluble polyphenols were secondary to initial N concentration in the residue in predicting total N mineralized.

Because of the increasing need to utilize animal manures and composts more efficiently, many researchers have studied the properties that affect C and N

mineralization in these amendments. Soil texture appears to play an important role in affecting mineralization rates. Castellanos and Pratt (1981) incubated several animal manures in a San Emigdio fine sand and Holtville silty clay, and reported more N mineralized in the fine sand for all types of manures. After incubating three broiler litters and two turkey litters on one loam and two loamy sand soils, Westerman et al. (1988) noted that more N was mineralized from the loamy sand soils for all litters. Sørensen and Jensen (1995) found increasing net N immobilization with increasing clay concentrations, and in a related study using N from sheep urine, found more N immobilization in a sandy loam compared to a sandy soil. Gordillo and Cabrera (1997) found the lowest net N mineralized in the soils of highest silt and clay concentrations, and highest N mineralized in soils with the second highest sand concentrations, after incubating nine soils of various textures with broiler litter. Egelkraut et al. (2000) reported that soils with higher clay concentrations had longer time periods of initial N immobilization and mineralized less N from added residues than those with lower clay concentrations. Ladd et al. (1981), Sorensen (1983a,b), Merx et al. (1985), Ladd and Amato (1988), and Voroney et al. (1989) also reported on the stabilizing effect of clay particles. The increased stability of soil organic matter in the presence of higher clay concentrations is due to its adsorption to charged surfaces (Greenland, 1965; Oades, 1988) and physical protection from microbial attack within small pores of microaggregates, which are more abundant in finer textured soils (Adu and Oades, 1978; Young and Spycher, 1979; Elliot, 1986; Gregorich et al., 1989). Zagal (1992) noted that microbial biomass concentration is related to soil texture and presumed it was because higher clay concentrations increased the ability of soils to preserve microbial biomass. However, Strong et al. (1999) stated that the protective



mechanisms of clay are undermined when a soil is dried and rewetted. They believe this may be due to the clay limiting the diffusion of partially decomposed organic materials away from the microbial decomposer population, facilitating more complete decomposition of the organic matter.

Nitrogen mineralized from soil organic matter depends to a certain degree on soil aggregate and particle size. Edwards and Bremner (1967) and Craswell et al. (1970) suggest that soil organic matter may become inaccessible to microbial attack as soil microaggregates are formed. Beare et al. (1994) indicated that macroaggregates in no-till soil are an important mechanism for the protection of SOM that would otherwise mineralize under conventional-till practices. Waring and Bremner (1964) noted increased N mineralization rates with decreasing soil mesh size. Craswell and Waring (1972a,b) ground soils to reduce aggregate size and observed higher N mineralization rates. Hiura et al. (1976) suggested using the clay/humus ratio as an index of potentially available organic N to microorganisms. Cabrera and Kissel (1988b) proposed the clay/total N ratio of a soil to estimate the degree of protection clays provide organic matter against microbial attack. Chichester (1969), McKeague (1971), and Cameron and Posner (1979), conducted experiments that separated soils by particle size, and reported decreasing particle size correlated with lower C:N ratios of soil organic matter, and that the N mineralized as a percent of the total N of that fraction increased with decreasing particle size. Cameron and Posner concluded this was primarily due to the fact that most of the readily mineralizable N resided in mineral fractions of less than 4  $\mu\text{m}$ , and much of this was recently formed microbial tissues or metabolites.

### Effect of moisture on C and N mineralization

The effect soil water content/potential on C and N mineralization from soil and decomposing plant residues has been studied for some time. Greaves and Carter (1920) noted that soils differing in relative saturation produced dramatically different levels of nitrate after 21 days of incubation. Russell et al. (1925) found insignificant levels of  $\text{NO}_3\text{-N}$  in soils that were at the “hygroscopic coefficient”, but increasing amounts at the highest level of moisture they studied, which was 1.25 times the “moisture equivalent” or field capacity. More recently, Quemada and Cabrera (1997) showed that as soil matric potential ( $\psi$ ) increased from  $-5.0$  to  $-0.003$  MPa the total  $\text{CO}_2$  evolved from unamended soil increased linearly with  $\ln(-\psi)$ , but  $\text{CO}_2$  evolved from decomposing clover residue increased exponentially with  $\psi$ . By contrast, using undisturbed soils in laboratory incubations, Sierra (1997) found that the rate of N mineralization showed a high degree of variability, with 57% of the variation associated with factors other than moisture and temperature. In field studies that confirmed the importance of moisture studies conducted in the laboratory, Powers (1990) showed that soil temperature and moisture strongly controlled the amount of N mineralized.

In a 30-week aerobic incubation study (Stanford and Hanway, 1955; Legg et al., 1971), Stanford and Smith (1972) proposed the N mineralization potential ( $N_o$ ) can be estimated from the equation:  $\log(N_o - N_t) = \log N_o - k \cdot t / 2.303$  or rearranged:  $N_t = N_o(1 - e^{-kt})$  where  $N_t$  is cumulative N mineralized at time  $t$ ,  $N_o$  is mineralized N at time zero,  $t$  is incubation time and  $k$  is the mineralization rate constant. Developing this work further, Stanford et al. (1973) determined the rate constant  $k$  had a  $Q_{10}$  of approximately 2 within the temperature range of 5-35° C. Maximum rates of N mineralization were reported by

Stanford and Epstein (1974) with soil moisture potential between  $-10$  to  $-33$  kPa for nine different soils, then proposed a method to model the influence of soil water concentration on N mineralization. The model normalized the various optimum water concentrations of the soils, verified that the regression coefficients of N mineralized on soil water concentration for the nine soils did not differ significantly (99% probability), then derived the equation:  $Y = -3.9 + 1.02X$  ( $r^2=0.93$ ), where  $Y$  denotes relative N mineralized and  $X$  denotes relative soil water concentration. Myers et al. (1982) modified Stanford and Smith's equation to express net nitrogen mineralized as a proportion of the maximum rate versus a normalized moisture concentration fitted between  $-0.03$  and  $-4.0$  MPa. Pilbeam et al. (1993) pretreated soils at different moisture concentrations for 24 days. They measured, over a period of nine days, increased rates of N mineralization in soils pretreated at higher moisture levels than lower moisture levels.

Cook and Allan (1992a) studied dissolved organic carbon (DOC) over 210 days in soils maintained at a constant moisture, and found asymptotic exponential response curves described positive associations between DOC and  $\text{CO}_2\text{-C}$  mineralization rates at early (14 and 35d) incubation times, but not later. The results reflected decreased DOC utilization relative to supply, and suggested this could be caused by the accumulation of recalcitrant DOC. In a related paper (Cook and Allan, 1992b) they determined, using the Leenheer DOC fractionation scheme, that although they could not directly determine DOC utilization, their results demonstrated that soil DOC was altered during the decomposition of soil organic matter.

### **Effect of wetting and drying on C and N mineralization**

Physically drying, then rewetting a soil may increase the rate of C and N mineralization. Birch (1958) conducted several experiments in which he demonstrated that when a soil was dried and rewetted, a flux of mineral carbon and nitrogen was observed immediately after wetting. In earlier work Birch and Friend (1956) concluded that successive wetting and drying released small amounts of decomposable material from within the clay lattice where it had been protected from microbial attack. This view was further developed to include the idea that a flush of decomposition was also due to the high activity of the newly developing microbial population and the amount of decomposable organic material that goes into solution (Birch 1959). Birch also provided evidence to show that the amount of C mineralized was proportional to the C concentration of the soil (Birch, 1960). Soulides and Allison (1961) found that prolonged drying increased the rate of decomposition of the soil organic matter, and that multiple drying events had a cumulative effect. A larger proportion of the bacteria died after the first and second drying than after the third and fourth, inferring that younger cells were killed more easily than older ones that were in a resting or sporulated stage. Stevenson (1956), and Hayashi and Harada (1969) also reported the drying process resulted in the killing of microorganisms. Marumoto et al. (1977a,b) concluded that dead microbial cells and their cell walls were a major source of the decomposable soil organic matter. Research conducted subsequent to Birch's work in the late 1950's confirmed that periodic wetting and drying is correlated to higher CO<sub>2</sub> production (Jager and Bruins, 1975; Herlihy, 1979).

Periodic drying and rewetting does not produce increased C and N mineralization over soils kept continuously moist in all cases. Van Schreven (1967) noted that intermittent drying at 35° C initially produced larger amounts of CO<sub>2</sub>, but less overall compared to treatments kept continuously moist, when calculated over the entire 12-week length of the experiment. He also indicated that the increase in the number of microorganisms persisted days and weeks longer than the increase in CO<sub>2</sub> production, inferring that the younger bacterial population was the most active. Franzluebbers et al. (1994) and Curtin et al. (1998) reported decreased C and N mineralization rates for soils subjected to repeated wetting and drying compared to soils kept continuously moist. White et al. (1998) reported decreased biodegradability, extractability, and uptake of organic compounds such as phenanthrene and DEHP by earthworms in soils subjected to repeated wetting and drying compared to soils kept constantly moist. However, based on field and microcosm studies, Lundquist et al. (1999b) stated that the increase in dissolved organic carbon (DOC) in soils exposed to wet-dry cycles indicates that wet-dry cycles contribute to higher DOC concentrations in the soil solution. Baldwin and Mitchell (2000) make the point that the extent of drying may determine whether more or less N is mineralized. Partial drying of wet sediments may reduce N availability by producing a zone for nitrification coupled with denitrification, while complete desiccation of the soil may lead to the death of bacteria and subsequent mineralization of N.

Cabrera (1993) successfully modeled the flush of N mineralization that follows rewetting, fitting the release to a modified Stanford and Smith (1972) equation, e.g.  $N_t = N_o (1 - e^{-k_1 t}) + k_o t$ , where  $k_1$  and  $k_o$  are mineralization rate constants, and  $N_o$ ,  $N_t$ , and  $t$  are potential mineralizable N, cumulative N mineralized at time  $t$ , and incubation time,

respectively. He suggested the first-order N flush was superimposed on zero-order background mineralization. In experiments utilizing  $^{14}\text{C}$ , Van Gestel et al. (1993b) fit first order decay rates to biomass  $^{14}\text{C}$  and nonbiomass  $^{14}\text{C}$ . They concluded that soil drying and rewetting promoted the turnover of C derived from added plant material, and that this increase in C cycling was mainly due to enhanced turnover of microbial products.

The effort to determine the proportional contributions of soil organic matter and recently added plant residues to C and N mineralization has yielded unclear results. Sørensen (1974) measured the  $\text{CO}_2$  release from  $^{14}\text{C}$  labelled glucose, cellulose, and straw added to soil that was allowed to metabolize for a period ranging from 1.5 to 8 years. Air-drying and rewetting every 30 days over incubation periods of 260 and 500 days produced  $\text{CO}_2$  levels 16 to 121% higher than controls maintained at a constant moisture level. Some of the difficulty in partitioning soil organic matter-C and plant residue-C can be ascribed to the “priming effect” reviewed by Jenkinson (1966a). The priming effect states that the addition of plant residue to a soil can increase the mineralization rate of soil organic matter as compared to controls without addition of plant residue (Sørensen, 1974).

### **Carbon and nitrogen mineralization rates based on microbial biomass response to repeated wetting and drying**

Seneviratne and Wild (1985) attributed the effect of drying on nitrogen mineralization to two causes: the death and subsequent lysis of a small proportion of the soil organisms, and desorption of organic substances with a wide C/N ratio. Van Gestel

et al. (1993a) inferred that the sources of mineralization flushes were partly biomass killed by drying, and partly non-microbial organic residues, and that the size of the flushes seemed to be influenced by soil properties such as C concentration and soil texture.

Kieft et al. (1987) demonstrated that most microbial biomass lysis occurred at rewetting, not during the slow drying period, explaining that slow drying allows microbes to adjust osmotic potential to their surrounding environment, but rapid rewetting causes cell rupture. Cortez (1989) noted that not all soil biomass undergoes lysis during rewetting, suggesting the presence of an active and a dormant fraction of soil biomass. De Bruin et al. (1989) postulated that the only microbial growth taking place after 30 days in a rewetted soil was based on the turnover of C and N from the microbial biomass itself. Bloem et al. (1992) utilized direct microscopy to note that while bacterial numbers did not change significantly, O<sub>2</sub> consumption and N mineralization decreased during a drying period, yet respiration increased up to 1.5 fold and N mineralization increased up to 5 fold following rewetting. This was attributed to the frequency of dividing-divided cells (FDDC), which increased up to 23% over soils maintained at constant moisture. Stark and Firestone (1995) demonstrated the relative importance of microbial cell osmotic potential and substrate diffusion in a drying soil on microbial activity, finding that substrate limitation was the major inhibiting factor when soil water potentials were greater than -0.6 MPa, but adverse physiologic effects due to cell dehydration was more significant in soils with water potentials less than -0.6 MPa. In a field study investigating differences in conventional, low-input, and organic farming systems, Lundquist et al. (1999b) suggested surface microbial populations had adapted to wet/dry

cycles over a three month growing season, leading to changes in microbial process rates and community composition. Kuikman et al. (1991) pointed out that protozoa play a role in N mineralization by reducing the number of bacteria and increasing the mineral N concentration in the soil. In experiments that contrasted continuously moist soils with those allowed to fluctuate, they showed that protozoa stimulated the mineralization of N 5-10% compared to soils without protozoa.

### **Compost and leaf litter in C and N mineralization**

As crop residues decay, they form an important component of organic C and organic N in the soil. Portions of these organic compounds are mineralized over time to provide a source of energy to soil flora and fauna. Several studies have utilized crop residues or composts to observe rates of C and N mineralization in soils.

Appel and Mengel (1993) reported decomposition and mineralization of sugar beet leaves incorporated into soils enhanced available N for plants, reflected by an increase of inorganic soil N, as well as of extractable organic N fractions. They concluded from their study on sandy soils that the extractable soil organic N fractions reflect the soils' microbial activities, and represent easily-mineralizable N pools in sandy soils.

Franzleubbers et al. (1995) reported soils with microbial biomass ranging from <10 to 1042-mg C kg<sup>-1</sup>; soil organic C ranged from 2.0 to 13.7-mg kg<sup>-1</sup>. Cowpea leaves incorporated into the soil had 458-g C kg<sup>-1</sup>, and a N concentration of 36-g kg<sup>-1</sup>, for a C:N ratio of 12.7. While initial C and N mineralization rates in a subsoil were less than topsoil treatments, at the end of 60 days total C and N mineralization were not significantly different, indicating microbial biomass adjusted quickly to the new substrate, regardless



of preexisting conditions. In a study of cotton grown on non-irrigated Ultisols, Mullins and Burmester (1990) found cotton leaf dry matter production to range between 942 and 1466 kg ha<sup>-1</sup>.

Airan and Bell (1980) define composting as a process for decomposition of organic solid wastes into relatively stable humus-like materials, and that the decomposition process is accomplished by various microorganisms including bacteria, actinomycetes, and fungi. He et al. (1992) note that when discussing municipal solid wastes (MSW) various operational parameters, such as source and nature of the raw material, source separation, composting temperature, moisture content, degree of aeration, and composting duration can significantly affect compost properties. While acknowledging the difficulty in making a general description of all MSW composts, they note that the moisture content of most composts varies between 20 and 50%, ash content is about 50%, C content is 30% on a dry-weight basis, and pH is generally neutral or slightly higher. De Haan (1981) found that while MSW compost is higher in N and P than most agricultural soils, only 10 to 15% of the total N is available the first year, and no residual effect remains in the second year. Navarro et al. (1992) applied 2.7 and 9.0-g MSW compost per 100-g soil, calculated according to the N needs of rye grass, assuming 50% N mineralization in the lower rate. "Mature compost" (not defined) contained 27.1% C, 1.5% N, and a C:N ratio of 18.1. Total organic N in the soil (soil not classified) ranged from 0.11 to 0.31-g N per 100-g soil. They found initial N immobilization, lasting three to five weeks, in a study conducted at field capacity and constant temperature (28° C). Sikora and Yakovchenko (1996) used a MSW compost that contained 146-g C kg<sup>-1</sup>, 12-g N kg<sup>-1</sup>, and a C:N ratio of 12.3, to study soil organic matter decomposition after

amending with compost. They conclude that if a priming factor exists, as discussed earlier, it is of low magnitude, short duration, and would not be a significant benefit in providing mineral N to plants. Their data suggests that the beneficial effects of adding composts to soil are not due to increased mineralization of SOM C or N within the first 1440 hours. The benefits of adding compost to a soil may be more related to improved soil physical properties, such as reduced compaction and higher water holding capacity. Mamo et al. (1999) utilized two different composts over three years with organic carbon ranging from 17 to 24%, N from 0.9 to 1.1%, and C:N ratios from 15 to 27. They applied this material at rates equivalent to 90 to 270 Mg ha<sup>-1</sup>. At the end of the 64-day incubation period the composts mineralized less than 0.2% of the total soil N, while N mineralized in the control soil was 1.6% of the total soil N. Egelkraut et al. (2000) used a MSW compost and cotton leaves and stems as incubation materials in a study comparing C and N mineralization in soils differing in texture. The compost contained 36.74% C, 12.74% organic N, and a C:N ratio of 28.8. The cotton leaves were 43.84% C, 28.3% organic N, and had a 15.5 C:N ratio. The compost had net N immobilization for the first 47 to 131 days of the study, depending on soil type. While cotton leaves initially immobilized N, all soil types had net mineral N accumulations after 7 days, with increasing N mineralization until the 179 day end of the study.

In contrast to MSW composts, composted dairy manures released 11 to 29% of their total N content as inorganic N after 32 weeks. Composted manure contained, on average, 33.2% organic matter and 2.4% N (Hadas and Portnoy, 1994). They noted that while no immobilization occurred, N mineralization rates were suppressed compared to the controls during the first week. They postulate that this is due to N assimilation by

microbes, as evidenced by high initial CO<sub>2</sub> rates. In a field study on the effect of applying MSW compost (C:N ratio 40:1) at different rates, Eriksen et al. (1999) observed that soil NO<sub>3</sub>-N was inversely proportional to MSW compost rates the first year. In the second year, though, there was an increasing supply of plant-available N, due to mineralization of organic N in the MSW compost with increasing MSW compost rate; however, the supply of mineralized N was inadequate to meet crop growth requirements for maximum maize (*Zea mays*) yield.

**CHAPTER 1**  
**PRELIMINARY WORK TO STUDY THE EFFECT OF DRYING ON C AND N**  
**MINERALIZATION**

**Introduction**

In order to quantify the effects of drying on C and N mineralization, an experimental system was needed that would either allow relatively rapid drying of soil or maintain soil moisture, while in both cases providing sufficient oxygen to maintain normal respiration rates. Preliminary studies were conducted to ascertain whether an aquarium system would fulfill the requirements of the experiment, and to ensure mineralized C and N were collected accurately without interfering with, or causing damage to, the system. These studies included placing pre-moistened soil treatments in the aquarium to determine moisture retention over time; quantifying the volume of leachate needed to extract mineralized N on a periodic basis; observing CO<sub>2</sub> evolution from treatments in order to determine best sampling times; and gathering rainfall data from the Georgia Coastal Plain to determine the length of the drying period for the soil treatments.

**Materials and Methods**

Our objective was to study the effect of wetting and drying on N mineralization. Preliminary studies were carried out to determine the time required to dry soil from field capacity to air-dry. Treatments were separated into two (25.5 x 51 x 30.5-cm) aquariums,

with one aquarium maintained at a constant, moisture-saturated humidity, and the other maintained at the humidity of the laboratory air. Each aquarium was covered in black duct tape to prevent light penetration, in order to avoid algae growth. A 31-L aquarium was selected for this purpose, modified with stainless steel lids, with 4-mm air-entry and air-exit ports, covering the tanks. Air was pumped in by means of two Second Nature® Challenger 1™ aquarium air pumps that forced 4-L air minute<sup>-1</sup> through Nalgene® (Nalge Nunc International Corporation, Rochester, NY) premium, Grade VI, (1/8" ID) tubing, 60-cm long into either a carboy partially filled with 4-L deionized water, or a dry glass bottle, depending on the treatment. For the wet treatment the air continued down a tube until it bubbled into the deionized water in the bottom of the carboy. Moisture saturated air then escaped at the top of the carboy through a 90-cm long, Nalgene® premium, Grade VI, (1/8" ID) tube into the aquarium. The aquarium for the wet treatment contained a 2-cm layer of deionized water in the bottom of the tank, while the aquarium for the dry treatment did not. For the dry treatment, air was pumped from the dry glass bottle through a 90-cm tube into the aquarium. Each aquarium contained two removable, porous, stainless steel shelves to hold the soil containers.

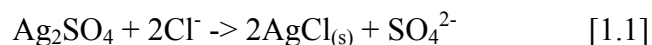
Experimental units consisted of 100-g oven-dry equivalent soil placed in a Nalgene® 70-mm diameter, polypropylene Buchner funnel top (funnel portion removed), after a Supor®-450 (Pall Gelman Sciences, Ann Arbor, MI) 0.45µm polymer filter was placed in the bottom of the Buchner funnel top. Triplicate replications of all treatments were brought to field capacity (-0.01 MPa) with deionized water and placed in their respective aquariums, and weighed daily to the nearest 0.1g, on an O'Haus Model No. AP210-0 scale (O'Haus Corporation, Florham Park, NJ), for eleven days. The soils

utilized were a Norfolk loamy sand, (coarse-loamy, kaolinitic, thermic Typic Kandiudults), and an Orangeburg sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudults), that were air-dried, crushed, and passed through a 2-mm sieve to remove small rocks and crop residues (< 1% of soil weight).

Soil treatments were brought to field capacity by placing 100-g oven-dry equivalent soil in the Buchner funnel attached to a vacuum source, first covering the floor of the funnel with a 0.45- $\mu\text{m}$  polymer filter paper. The soil was smoothed and gently tamped down with the bottom of a volumetric flask. A Whatman #42 filter paper was custom cut to completely cover the soil in the flask and placed on top of the soil. 60.0-mL deionized water – representing 15% moisture, which exceeds the known field capacity of these soils - was poured slowly over the top filter, taking care to thoroughly and evenly wet the filter. The deionized water was allowed to drain gravimetrically for 30 min. Vacuum was applied for 30 min and allowed to run until all excess moisture had drained. The funnel weights were weighed and recorded (Figure 1.1).

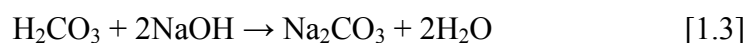
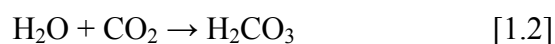
To determine an appropriate volume of leaching solution to remove  $\text{NO}_3\text{-N}$  from 100 g of residue treated soil in buchner funnels, a preliminary experiment was conducted using  $\text{Cl}^-$  ions as a substitute for  $\text{NO}_3\text{-N}$ , since  $\text{Cl}^-$  has been shown to move similarly to  $\text{NO}_3^-$  in soils (Nielsen and Biggar, 1961). Background soil  $\text{Cl}^-$  levels were determined by combining 100.00 g soil with 400 mL  $\text{H}_2\text{O}$  in a 600-mL beaker and titrating with 0.0065 M  $\text{Ag}_2\text{SO}_4$ . Three separate KCl solutions were formulated so that each soil could be brought to its field capacity with a KCl solution that would have 12.66 mg  $\text{Cl}^- \text{ kg}^{-1}$  soil. These solutions were pipetted into the appropriate soil to bring them to field capacity. They were allowed to equilibrate overnight in the moist-air aquarium, then were leached

with 50-mL aliquots of deionized H<sub>2</sub>O under vacuum. The 50-mL aliquots were titrated with 0.0065 M Ag<sub>2</sub>SO<sub>4</sub> to an endpoint of 290-mv with a Beckman 39048 silver electrode/calomel electrode (Beckman Instruments, Inc., Fullerton, California) in order to calculate percent chloride recovery (Figure 1.2). The reaction is:



One objective of the main experiment was to evaluate the rate of CO<sub>2</sub>-C mineralized from the treatments. To determine the appropriate measurement times, a preliminary experiment was conducted to measure the rate of CO<sub>2</sub> loss from three soils with two organic residues using an infrared gas analyzer (IRGA). Soils used were the Orangeburg, Norfolk, and Norfolk depressional, described earlier. 100.000 g of each soil were mixed thoroughly with either 0.2057 g ball-milled cotton leaves or 1.5005 g ball-milled compost, and placed in a 70-mm diameter Buchner funnel top with a 0.45 μm polymer filter. Soil in the funnels was brought to field capacity (-0.01 MPa) by pipetting appropriate quantities of deionized water. The base of the funnel was sealed with Saran® wrap. Lids made of Poly-vinyl Chloride that were machined for a rubber O-ring sealed the top. A rubber septum was placed in the middle of the PVC lid to allow air to be pumped through by syringe injection. Air was pumped using aquarium air pumps described earlier into a glass bottle containing M NaOH to scrub the CO<sub>2</sub> out of the air.

The reactions are:



The CO<sub>2</sub>-scrubbed air passed into the sealed treatment container head space. Air then passed into a Li-Cor IRGA CO<sub>2</sub> Analyzer, Model LI-6252 (Li-Cor, Inc., Lincoln, NE) which measures μmol CO<sub>2</sub> mol-air<sup>-1</sup>. Using a flow meter μg CO<sub>2</sub>-C g-soil<sup>-1</sup> hr<sup>-1</sup> were calculated according to the following equation:

$$(\text{Flow rate (mL min}^{-1}) \times 0.06) / 22.4 \times \text{IRGA reading (}\mu\text{mol CO}_2 \text{ mol air}^{-1}) \times 12 / \text{g soil} \quad [1.4]$$

where flow rate x 0.06 = liters of air hr<sup>-1</sup>; L hr<sup>-1</sup>/22.4 = mol air hr<sup>-1</sup> (22.4-L is volume of one mol air at standard temperature and pressure) The volume of air in liters was corrected for temperature and pressure as recorded during the experiment according to the formula given in the handbook of Chemistry and Physics, 45<sup>th</sup> edition, 1964,1965, which is:

$$\text{density of dry air (g mL}^{-1}) = (0.001293 / (1 + 0.00367t)) \times (H/76) \quad [1.5]$$

where *t* is temperature in degrees C, and *H* is pressure in cm mercury. The density of dry air was multiplied by 1000 to obtain g L<sup>-1</sup>. The formula weight of air, for the major



components N, O, CO<sub>2</sub>, and Ar, as calculated from the Handbook of Chemistry and Physics, 45<sup>th</sup> ed, is 28.953, so 28.593 g / the density of dry air (g L<sup>-1</sup>) gave L.

Readings were taken approximately every hour for the first 24 hours, with time between readings expanding as the project progressed.

Precipitation data was gathered from three research locations located in the Coastal Plain of Georgia. Data from the National Peanut Research Laboratory, U.S.D.A – A.R.S. near Dawson, The Coastal Plain Experiment Station, University of Georgia, near Tifton, and the Catahoula Farm, University of Georgia, near Cordele, was analyzed to determine the frequency of rain events, and the number of days between rain events, over a “growing season”. The growing season spanned March 15<sup>th</sup> to October 15<sup>th</sup>, to roughly coincide with average planting and harvesting dates for the area, with data covering the years 1997 through 2001. Rain events equaling or exceeding 0.25-cm day<sup>-1</sup> were enumerated, then totaled. The 215-day span covered was divided by the number of rain events for that season to obtain an average number of days between rains. For all locations covering all years the average number of days between rain events was 6.00 days with a standard deviation of 1.49.

Because rain events often clustered, a second method was used to evaluate days between rain events. The rationale was to calculate the mean number of consecutive days with no rain, so that it could be determined how many drying days the soil had on average. Days that contained less than 0.25-cm precipitation were counted as “dry” days. Using this method, the average number of consecutive days between rain events was 6.89. There was quite a bit of variability in the number of days between rains, for a given location over a given growing season, as the average standard deviation was 6.81. The

longest time span between rain events equaling or exceeding 0.25-cm was 70 days, occurring between July 2 and September 9<sup>th</sup>, 1997 at the Catahoula Farm near Cordele.

The average number of days between rain events was close to seven. The preliminary tests to dry the soil treatments revealed that it took at least eight days in the laboratory to reach air-dry. Therefore, it was decided for the main experiment to allow eight days to dry the soil to air-dry, then an additional three days to place the soil biota under stress, before rewetting.

### **Results and Discussion**

At field capacity (-0.01MPa) the Orangeburg soil held 0.103 g H<sub>2</sub>O per gram of soil, while the Norfolk held 0.080 g H<sub>2</sub>O per gram of soil. Over the study, treatments maintained in the moist environment (~98% RH) lost 0.00577 g H<sub>2</sub>O g<sup>-1</sup> Orangeburg soil or 0.58% of the total moisture, and 0.00398 g H<sub>2</sub>O g<sup>-1</sup> Norfolk soil or 4.98%. Treatments maintained in the laboratory-dry air (~50% RH) evaporated 0.0975 g H<sub>2</sub>O g<sup>-1</sup> Orangeburg soil or 94.66%, and 0.0771 g H<sub>2</sub>O g<sup>-1</sup> Norfolk soil or 96.38% (Figure 1.1). Most evaporation occurred the first eight days of the test, however it was presumed it would take longer to dry the treatments during the actual experiments due to an increased volume of treatment replications in the confined space of the aquariums. This preliminary test showed the forced-air aquariums were sufficient in maintaining adequate moisture in the moist-air tank, and sufficient in allowing soils at field capacity to evaporate moisture to air dryness (-100MPa).

In all soils and all replications, over 90% of the Cl<sup>-</sup> ions were recovered after 200 mL of deionized water were used to leach the treatments (Figure 1.2). The Norfolk soil

recovered 90.5% of chloride ions after four 50-ml aliquots of leachate, and 92.4% by five 50-mL aliquots. The Norfolk depressional recovered 93% of chloride by four 50-mL aliquots, and 94.1% by five 50-mL aliquots. The Orangeburg soil recovered 97.9% by four 50-mL aliquots, and 98.9% by five 50-mL aliquots. After 400-mL of leachate were extracted, the Orangeburg recovered 102.1% of chloride. A possible explanation for this is that the Orangeburg soil sample used, had more chloride in it than calculated from the soil extraction portion of the experiment. Based on these results, it was decided 250-mL leachate would be used in the main experiment in order to provide a margin of safety.

After integrating for total CO<sub>2</sub>-C mineralized, soils with cotton leaves mineralized an average of 109% more CO<sub>2</sub>-C than soils with compost residue. The Norfolk Depressional with cotton leaves evolved the most CO<sub>2</sub>-C at 0.257 mg CO<sub>2</sub>-C g soil<sup>-1</sup>. The least amount of C mineralized as CO<sub>2</sub> occurred in the Norfolk soil with compost at 0.069-mg CO<sub>2</sub>-C g soil<sup>-1</sup>. The study lasted 160 hr, with the majority of C released in the first 48 hr. The Orangeburg soil, with the greatest clay content of the three soils, but less soil organic matter than the Norfolk depressional, evolved 0.225 mg CO<sub>2</sub>-C g soil<sup>-1</sup> with cotton leaves, and 0.119 mg CO<sub>2</sub>-C g soil<sup>-1</sup> with compost. The soil with moderately high clay and highest organic matter of the three soils, released the most CO<sub>2</sub>-C, while the sandiest soil with the least soil organic matter released the least CO<sub>2</sub>-C (Figure 1.3). From this preliminary study of CO<sub>2</sub>-C mineralization, the decision was made to sample for CO<sub>2</sub> every 24 hours for two days after leaching, which rewet the soil, then at least every 48 hours until the next leaching event.

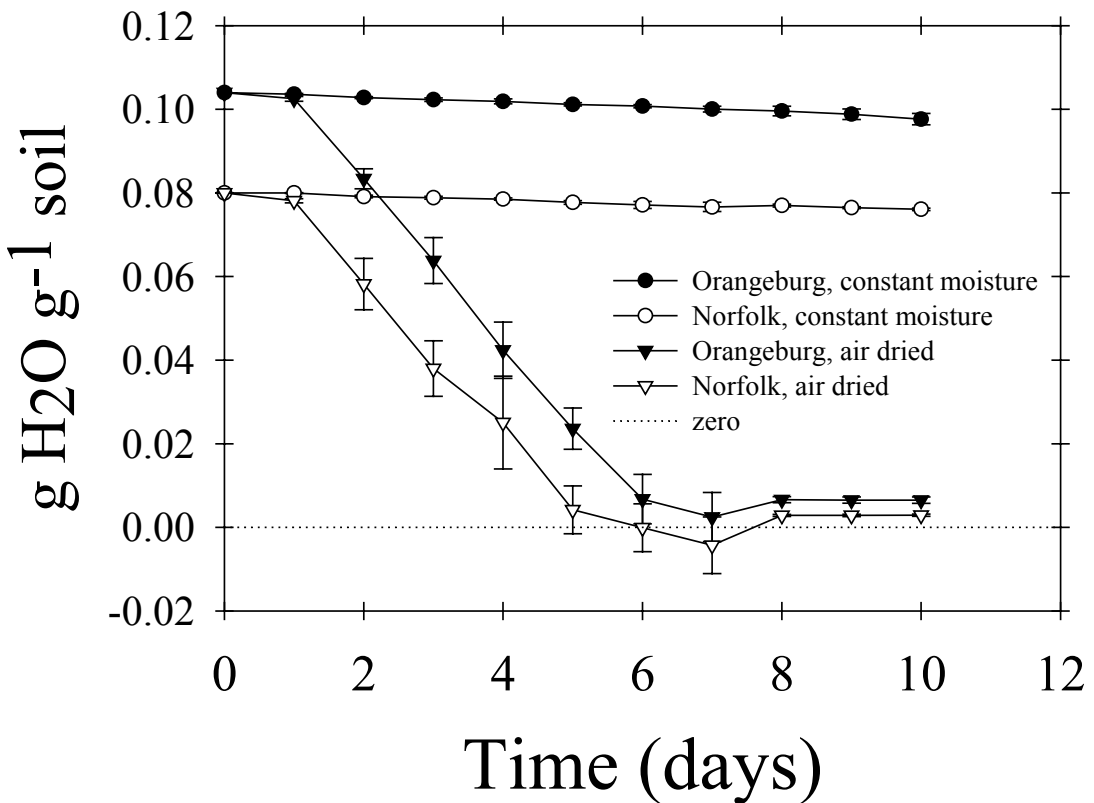


Figure 1.1 Loss of water content in Orangeburg and Norfolk soils in chambers maintained at 98 % and 50% RH.

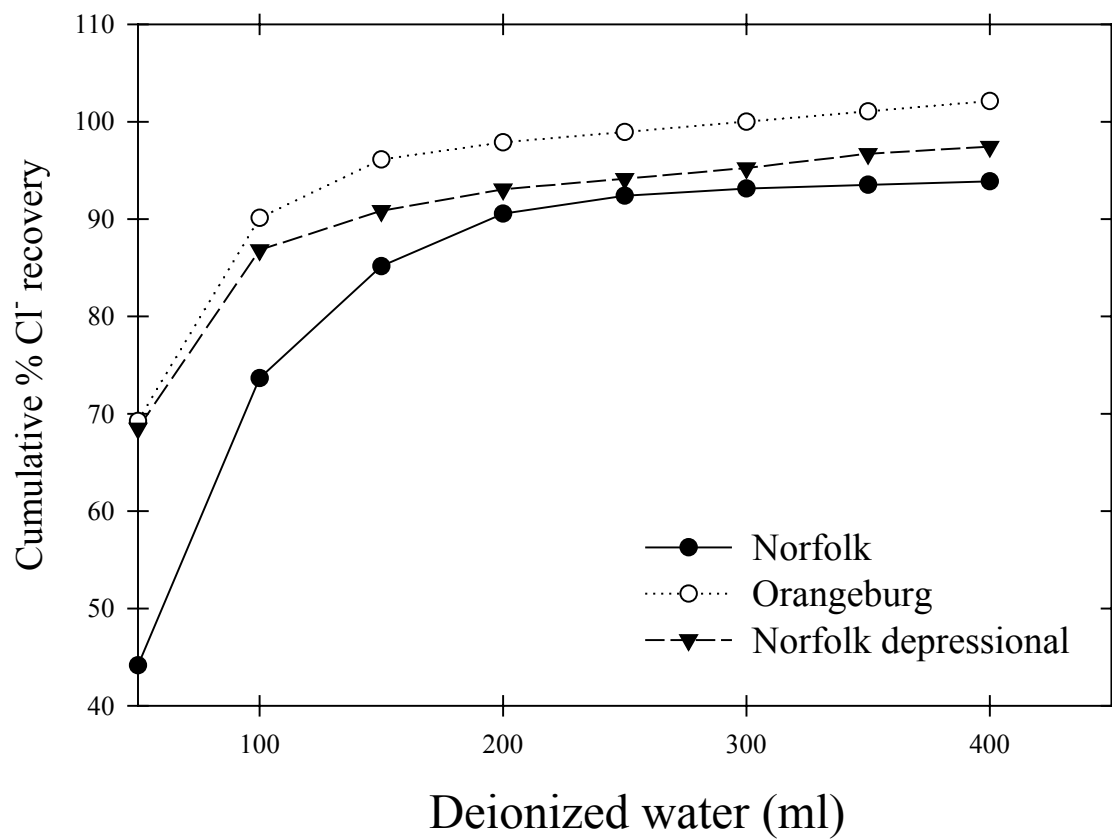


Figure 1.2 Cumulative %  $\text{Cl}^-$  recovery from coastal plain soils in 50 mL aliquots.

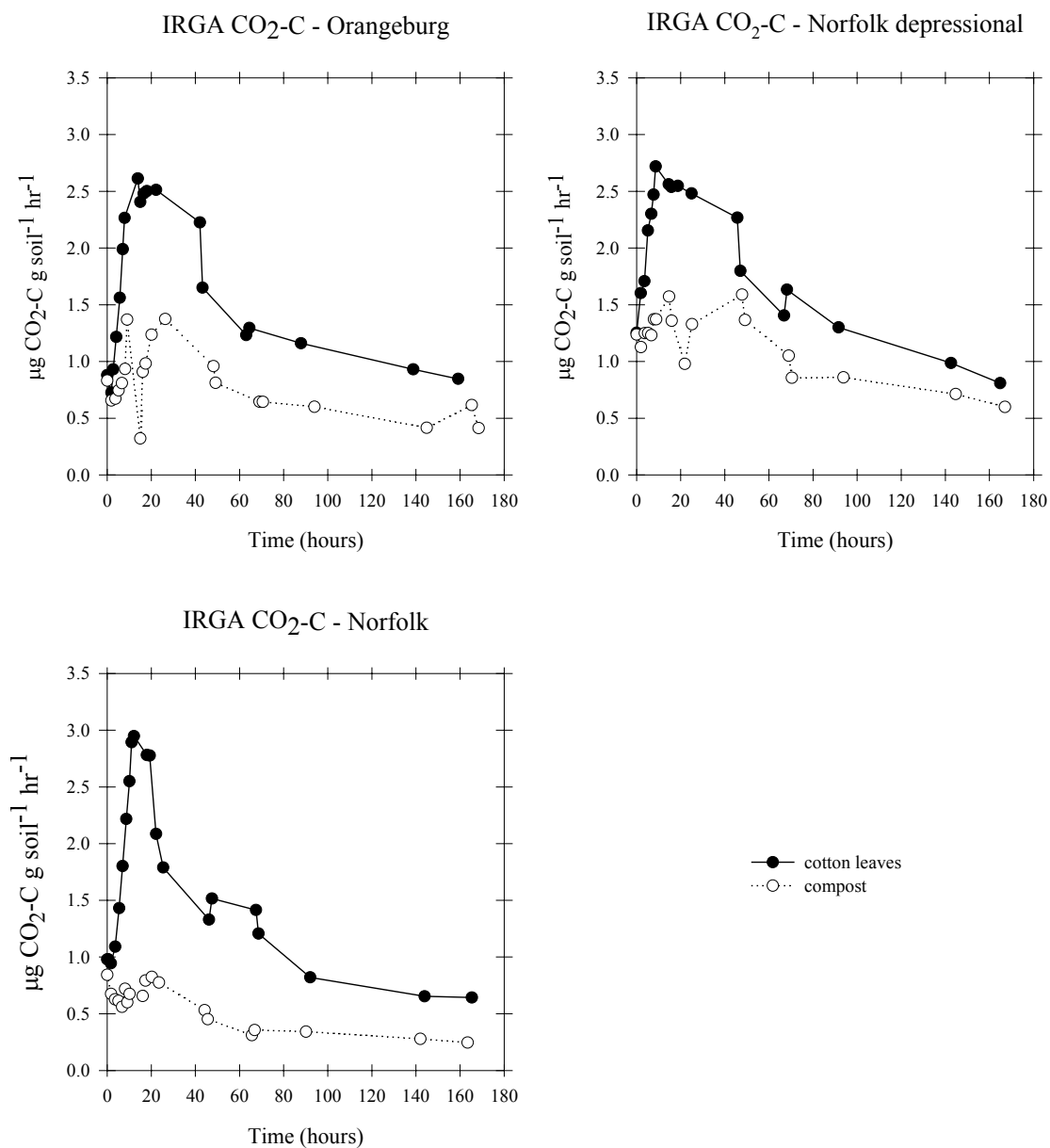


Figure 1.3 CO<sub>2</sub>-C evolved from coastal plain soils over 165 hours, as measured by infrared gas analyzer (IRGA), to determine initial rates of CO<sub>2</sub>-C evolution from soils with incorporated residues.

## **CHAPTER 2**

### **THE EFFECT OF WETTING AND DRYING ON NITROGEN MINERALIZATION**

#### **Introduction**

Many factors influence the rate of N mineralization in soils, including temperature, moisture, soil texture, soil organic matter, and crop residue composition. Some studies have focused on the effect moisture has on rates of N mineralization from soil organic matter (Stanford and Smith 1972, Stanford and Epstein 1974, Sierra 1997), while others have studied the effect moisture has on N mineralization from residues (Curtin et al. 1998, Das et al. 1993). Birch (1960) studied the effect wetting and drying had on N mineralization, with dry periods ranging from 3 to 15 weeks. Cabrera (1993) modeled the initial flush of N mineralization occurring hours after rewetting a dried soil.

The purpose of this study was to determine the effect repeated wetting and drying would have on the rate of N mineralization on coastal plain soils of the southeastern United States with different residues incorporated. The study was conducted over 185 d to simulate the approximate length of the growing season for a summer annual crop.

#### **Materials and Methods**

Three soil samples were collected from the upper 0.15 m of a field under conventional tillage in the coastal plain of Georgia to provide a range of clay and soil

organic matter contents. The Norfolk loamy sand (coarse-loamy, kaolinitic, thermic Typic Kandiudults) contained both the lowest clay percentage and the lowest soil organic matter. The Orangeburg sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudults) contained the highest clay content and the medium level of soil organic matter of the selected soils. The Norfolk depressional sandy loam (fine-loamy, kaolinitic, thermic Arenic Kandiudults), a taxadjunct to the Norfolk series, contained a medium clay level and the highest soil organic matter content of the three soils (Table 2.1). Soils were air dried in the laboratory, crushed, then sieved through a 2-mm sieve to remove small rocks and non-decayed crop residue, which constituted less than 1% of the soil by weight. The water content of the soils at field capacity ( $-0.01$  MPa) was determined by using a pressure chamber. Three replicates of each soil were placed into steel cores with a 2-cm radius and 0.9-cm height and saturated with deionized water at atmospheric pressure. The samples were placed into the pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA) and brought to  $-0.01$  MPa, weighed, dried in an oven at  $105^{\circ}\text{C}$  for 48 hours, then weighed again (Klute, 1986). A subsample of each soil was analyzed for C and N with a Carlo-Erba NA 1500 Analyzer for Carbon and Nitrogen, Milan, Italy, according to the methods described by Kirsten (1983).

The study treatment design was a factorial combination of three soils (Norfolk, Orangeburg, Norfolk depressional), three residues (none, cotton leaves, compost) and two soil water levels (constant wet and alternating wet and dry). Organic residues chosen for the study were cotton leaves and compost. The compost selected began as a mixture of grass clippings, hardwood leaves, and pine needles. Windrows were stacked 120 cm high and mixed until the materials went through a peak heat. Samples were sieved in a 2-mm



sieve, composited, and ball milled. A subsample was analyzed for C and N (Table 2.2) with a Carlo-Erba NA 1500 Analyzer for Carbon and Nitrogen. Cotton leaves were applied at 2057 mg kg<sup>-1</sup> soil, and compost was applied at 15000-mg kg<sup>-1</sup> soil. After placement of a 0.45- $\mu$ m Supor® 450 membrane filter (Gelman Sciences, Ann Arbor, MI) filter in the bottom of a (75-mm diameter x 40 mm height) buchner funnel, residues were mixed with soils by placing the weighed soil and residue into the buchner funnel, then stirring with a glass rod for 5 min. Soil that fell through the bottom of the buchner funnel was captured by placing the funnel on paper during stirring, then pouring the soil back into the buchner funnel. There were three replicates of all treatments.

Treatments were divided into two systems. Those maintained at field capacity for the duration of the study were kept in an aquarium (25.5 x 50 x 30.5 cm) whose external surface was taped with black duct tape to prevent algae growth. The bottom 2 cm of the tank held deionized water to help maintain high humidity. The top of the tank was covered by a stainless steel lid with 4-mm (inner diameter) entry and exit ports. Air was pumped in by means of two Second Nature® Challenger 1™ aquarium air pumps providing 4L min<sup>-1</sup> air through Tygon® tubing into the bottom of a carboy partially filled with 4L deionized water. Water-saturated air then exited through the top of the carboy via Tygon tubing into the entry port for the tank. Treatments that were dried and rewetted were stored in the high humidity tank for 3 d, then transferred to an identical tank that had laboratory air pumped into a dry glass bottle, then transferred into the entry port of the stainless steel lid. The bottom of the dry tank contained no water, but instead held 454 g Drierite® (W.A. Hammond Drierite Co., Xenia, OH) anhydrous calcium sulfate, that was changed daily. Soils were dried down in this tank for 11 d before being

rewetted. Temperature and relative humidity levels inside the tanks were recorded for both treatments two times per week.

Following the first 14-d cycle, all soils were weighed and the Buchner funnel tops containing the soil was attached to their funnel bottoms and inserted into a vacuum flask in preparation for leaching. A Whatman #42 filter paper disk was placed on top of the soil and 100 ml of 0.01M CaCl<sub>2</sub> was added, taking care to not disturb the soil, and allowing it to drain by gravity only. Then vacuum was applied, and more CaCl<sub>2</sub> was added in 50-mL aliquots, until a total of 200 mL was applied. This was followed by 50 mL of a N-free nutrient solution containing 430.48 mg L<sup>-1</sup> CaSO<sub>4</sub>, 243.41 mg L<sup>-1</sup> MgSO<sub>4</sub>, 2.19 mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, and 15.02 mg L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>. The leachate was stirred, transferred quantitatively, and brought to volume with 0.01M CaCl<sub>2</sub>. The samples were stored in a freezer at -20° C, until analyzed. After removal of the Whatman #42 filter paper disk, soil residue was scraped from the disk back into the treatment container. The 14-d incubation cycle was repeated a total of 13 times over 185 days. At the end of 185 days, all treatments were air-dried and weighed, then oven-dried and reweighed.

Nitrate was analyzed on a Dionex DX-120 IC (Sunnyvale, CA), which determined concentration based on electrical conductivity. Standards were made up in 0.01M CaCl<sub>2</sub>. Ammonium was analyzed on an OI Analytical Flow Solution 3000 (College Station, TX) using the automated phenate colorimetric procedure EPA-600/4-79-020, "Nitrogen, Ammonia" Method 350.1 (EPA, March 1984). Statistical analysis (GLM procedure) of the net N mineralized after 185 d of incubation was conducted using SAS (SAS Institute, 1985), followed by testing for mean separation, using Tukey's method.

Soluble organic C analysis was conducted by injecting 3 mL of the 0.01M CaCl<sub>2</sub> leachate through a 0.45 µm filter, then analyzed on a Shimadzu TOC-5050 (Shimadzu Corp., Kyoto) total organic C analyzer. The TOC-5050 combusts the sample and C is converted to CO<sub>2</sub>, which is then quantified by a non-dispersive infrared gas analyzer (NDIR), where CO<sub>2</sub> is detected. The NDIR outputs an analog signal, which generates a peak whose area is calculated by a data processor.

### **Results and Discussion**

In the control soils with no residue, the greatest amount of N mineralized came from the Norfolk depressional (Table 2.3). The Orangeburg and Norfolk mineralized statistically the same amount of N at  $\alpha = 0.05$ . By the end of the study, the control soils all mineralized between 4-8% of organic N present at the beginning of the study. The Orangeburg soil slightly immobilized N in the first 14 d; the Norfolk soil mineralized 18.8% of its total N mineralized, in the first 14 d; and the Norfolk depressional mineralized 39.1% of its total N mineralized, in the first 14 d (Figure 2.1). There was a steady increase in the cumulative N mineralized over time for all soils after 14 d (Figure 2.1). For control soils, the moisture regime did not significantly affect the rate of N mineralized at the  $\alpha = 0.05$  level for the soils (Table 2.3). The soil type was a significant factor at the  $\alpha = 0.05$  level, confirming previous work by Egelkraut et al. (2000). Tukey's method of mean separation revealed that the Norfolk depressional was different than the Norfolk and Orangeburg. A soil x moisture interaction was considered and analyzed with the GLM procedure, and it showed no significant interaction at the

$\alpha = 0.05$  level. Residual analysis was performed and showed the samples to be normally distributed, verifying the model.

Cumulative N mineralized, as % of N applied, for soils with incubation materials, is shown in Figure 2.2 for each of the moisture regimes. The GLM procedure was used to identify significant differences in % N mineralized from residues at the  $\alpha = 0.05$  level (Figure 2.4). Moisture regime was a significant factor, as were soil type and residue. Interactions were investigated and showed a significance for moisture x residue, but not for soil x moisture, soil x residue, or soil x moisture x residue. After means testing, using least square means, the moisture x residue interaction, it is apparent that when treatments are under constant moisture there is a significant difference between residues, but when treatments are repeatedly wetted and dried they are not significantly different (Figure 2.3). Residual analysis was performed and showed the sample data to be normally distributed, verifying the model (not shown).

For all soils, the most N mineralized after 185 d, as % of N applied, was from cotton leaves in the constant moisture regime. For all soils, cotton leaves displayed an initial period of N immobilization, as indicated by the negative portion of the curves in Figure 2.2, but showed faster net N mineralization in soils under a constant moisture regime than the soils that were subjected to repeated wetting and drying. The Orangeburg soil with cotton leaf residue that underwent repeated wetting and drying had no net N mineralization after 185 d.

At 185 d, for soils maintained at a constant moisture regime, the Orangeburg, Norfolk, and Norfolk depressional with cotton leaves had 25.7, 28.5, and 39.5% mineralized N as % applied, respectively. After 185 d, in soils with cotton leaves

undergoing repeated wetting and drying, the Norfolk soil had 2.0% mineralized N as % N applied, the Norfolk depressional had 6.85%, while the Orangeburg had 1.28 % immobilized N. There was a strong contrast in mineralization trends between soils with cotton leaves under a constant moisture regime versus repeated wetting and drying. While both sample populations initially immobilized N, those under constant moisture regime mineralized N by 45 d. By 58 d, 38-68% of the total N mineralized, had undergone mineralization. After 58 d, the rate of N mineralization appeared to slow down, as the remaining cotton leaf residue apparently became more difficult to break down, or microbial populations stabilized. Soils with cotton leaves subjected to repeated wetting and drying initially immobilized N, then mineralized N only slowly. At the end of 185 d, the Orangeburg still had net N immobilization, the Norfolk had 2% net N mineralization, and the Norfolk depressional 6.9% net N mineralization. The Norfolk had net N mineralization after 129 d, and the Norfolk depressional had net N mineralization after 58 d.

The cause of the difference in net N mineralization is not yet understood for this study. It is possible that the N not mineralized in the repeatedly wetted and dried treatments is still in the soils. It may be possible to verify this with a follow-up study incubating the treatments for 60 d under constant moisture conditions and then leach them with 1 M KCl. If, in the follow-up incubation study, the treatments that were under fluctuating moisture conditions in the initial study, mineralize significantly more N than the treatments that were under constant moisture in the initial study, it may indicate the N in the repeatedly wetted and dried soils did not mineralize much N in the initial study. Another possibility was that some N denitrified during the rewetting procedure. If

organic C analysis of the leachates revealed higher total C in the wetted and dried treatments than the constant moisture treatments, it may have been an indication that denitrification occurred. The presence of higher amounts of soluble C in the leachate, coupled with less mineral N in the leachate, could indicate the presence of denitrifiers, since denitrifiers would contain soluble C, but would have evolved N as N<sub>2</sub>O gas (Cabrera M.L., personal communication, 2002). An analysis of soluble organic C in the 0.01M CaCl<sub>2</sub> leachate revealed, however, less C in the repeatedly wetted and dried soils than those maintained at a constant moisture level (Figure 2.4).

Soils with compost either did not have any initial net N immobilization, or it was slight (<2%) and only lasted 14 d. The amount of N mineralized by 185 d was relatively small, regardless of moisture regime. Soils with compost maintained at a constant moisture level mineralized 3.8 to 9.3% of the N applied as compost, and soils that underwent repeated wetting and drying mineralized 1.6 to 3.3% of the N applied. There was a significant difference in N mineralized ( $\alpha=0.05$ ) between moisture regimes for the Orangeburg and Norfolk depressional soils, but not the Norfolk soil (Table 2.3). There was no significant difference in the amount of N mineralized between soil types ( $\alpha=0.05$ ) for soils treated with compost. The Orangeburg and Norfolk soils with compost maintained under constant moisture did not immobilize N. The Norfolk depressional with compost initially immobilized N, but had net N mineralization by 32 d, and mineralized more N than the other two soils as %N applied. The Norfolk depressional immobilized the most N of the soils with compost (1.9% of the compost N after 14 d). The net amount of N mineralized from compost by 185 d was 9.3% for the Norfolk depressional under constant moisture, 3.2% for the Norfolk depressional repeatedly

wetted and dried, 3.8% for the Norfolk under constant moisture, 1.7% for the Norfolk repeatedly wetted and dried, 4.9% for the Orangeburg under constant moisture, and 1.6% for the Orangeburg repeatedly wetted and dried (Table 2.3). These values are in line with previous results obtained by Tyson and Cabrera (1993), that showed 0.4 – 5.8% mineralized N in composted broiler litter.

It may be of interest to note that although there was a significant difference in the amount of N mineralized between the cotton leaves and compost (Table 2.3), there was not much difference between the C to N ratios of the two residues (Table 2.2). While the Orangeburg soil mineralized, in almost all cases, the least amount of N from the residues, it had the lowest C to N ratio of the three soils (Table 2.1). This may suggest that the C to N ratio of the soil organic matter had an impact on the quantity of N mineralized from residues. The higher C to N ratio of the Norfolk depressional soil, compared to the orangeburg soil, may indicate a higher C to N ratio in the base microbial population in the Norfolk depressional. A microbial population with a higher C to N ratio would require less N and therefore mineralize more N from the applied residues.

For all soils and all residues there was a significant difference between treatments maintained at constant moisture versus treatments subjected to repeated wetting and drying at  $\alpha=0.05$ . Soils maintained at constant moisture exhibited more N mineralized than those wetted and dried. The mean N mineralized for all soils with compost under constant moisture was 5.99%, while the mean N for all soils with compost repeatedly wetted and dried was 2.18% (LSD = 2.71). The difference for all soils with cotton leaves was more dramatic, with the mean N mineralized for all soils with cotton leaves under constant moisture was 31.23%, while the mean N mineralized for all soils with cotton

leaves repeatedly wetted and dried was 2.54% (LSD = 9.56). The initial amount of inorganic N in the compost was equivalent to 14.38 mg N kg<sup>-1</sup> soil, and the initial amount of inorganic N in the cotton leaves was equivalent to 1.17 mg N kg<sup>-1</sup> soil.

In Stanford and Epsteins' study of the relationship between soil water content and nitrogen mineralization (Stanford and Epstein, 1974), they established that at 35° C, the relative rate of N mineralization was equal to the soil water content / optimum soil water content. For example, at 40% of optimum water content (optimum water content was noted as 1/3 bar), one would observe 40% of the mineralization rate as at 100% optimum water content. The average soil moisture for the soils repeatedly wetted and dried was 49.9% over the length of the study. Based on Stanford and Epstein's work, one would expect roughly half the N to mineralize compared to the soils maintained at field capacity. For all control soils with no residue, there was no significant difference between N mineralized under optimum water conditions and N mineralized under repeatedly wet and dry conditions. This may suggest rewetting had a mechanistic or biological effect that overcame periods of dry soil with low biological activity and ensuing low N mineralization. Kieft et al. (1987) noted an increase in biomass-C release as inorganic C, following rapid rewetting, and a greater proportion of biomass-C release after a 6.9MPa increase compared to a 2.8 MPa increase. They concluded water potential increases associated with the wetting of a dry soil may be a major catalyst for soil C turnover. It can be inferred that a proportional amount of N, based on the C:N ratio of the biomass, would also release upon cell lysis.



Table 2.1 Soil particle size distribution, C and N concentration, C to N ratio, and initial inorganic N concentration

Soil	Clay -----%-----	Sand	Total C -----mg kg <sup>-1</sup> -----	Total N	C/N	Initial inorganic N mg kg <sup>-1</sup>
Orangeburg	19.6	72.4	3691	380	9.7	9.1
Norfolk	8.5	87.5	4381	322	13.6	1.4
Norfolk depressional	14.3	65.2	12526	753	16.6	13.1

Table 2.2 Dry weights, total C and N concentration, C to N ratio, and initial inorganic N concentration of cotton leaves and compost

Residues	Rate	Total C	Total N	Organic N	C/N	Initial inorganic N
	mg kg <sup>-1</sup>	-----g kg <sup>-1</sup> -----				mg kg <sup>-1</sup>
cotton leaves	2057	432.3	29.0	28.4	14.9	570.5
compost	15000	240.1	14.2	13.2	16.9	958.6

Table 2.3 Net N mineralization after 185 days of incubation for the three study soils and treatments

Soil and moisture regime	Leaves	Compost	Control
	% of applied N		mg kg <sup>-1</sup>
Orangeburg, constant moisture	25.72	4.86	16.09
Orangeburg, wet and dry	-1.28	1.59	18.32
Norfolk, constant moisture	28.45	3.78	23.10
Norfolk, wet and dry	2.04	1.69	21.02
Norfolk depressional, constant moisture	39.53	9.31	46.53
Norfolk depressional, wet and dry	6.85	3.25	40.32
LSD (a = 0.05) for moisture regime	9.56	2.71	6.45
LSD (a = 0.05) for soil type	N.S.	N.S.	5.29

Table 2.4 GLM Table for N mineralized as % N applied as residue.  $\alpha=0.05$ 

Source	df	SS	MS	F	Pr > F
soil	2	335.282	167.641	3.97	0.0294
moisture	1	2377.327	2377.327	56.36	<0.0001
residue	1	1475.156	1475.156	34.97	<0.0001
moisture x residue	1	1393.514	1393.514	33.04	<0.0001
error	30	1265.487	42.183		
Total	35	6846.766			

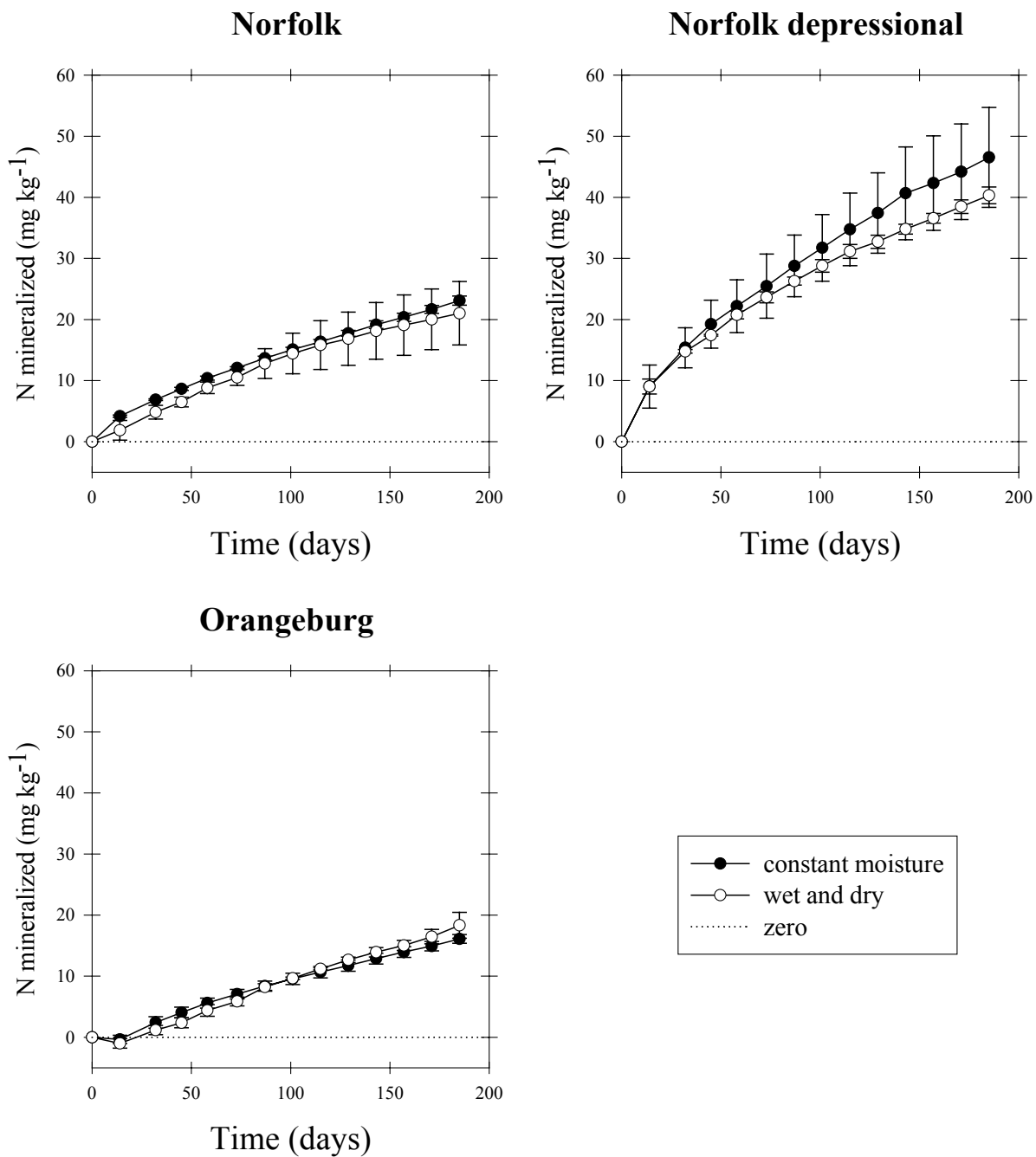


Figure 2.1 Cumulative N mineralized from the control soils with no residue.

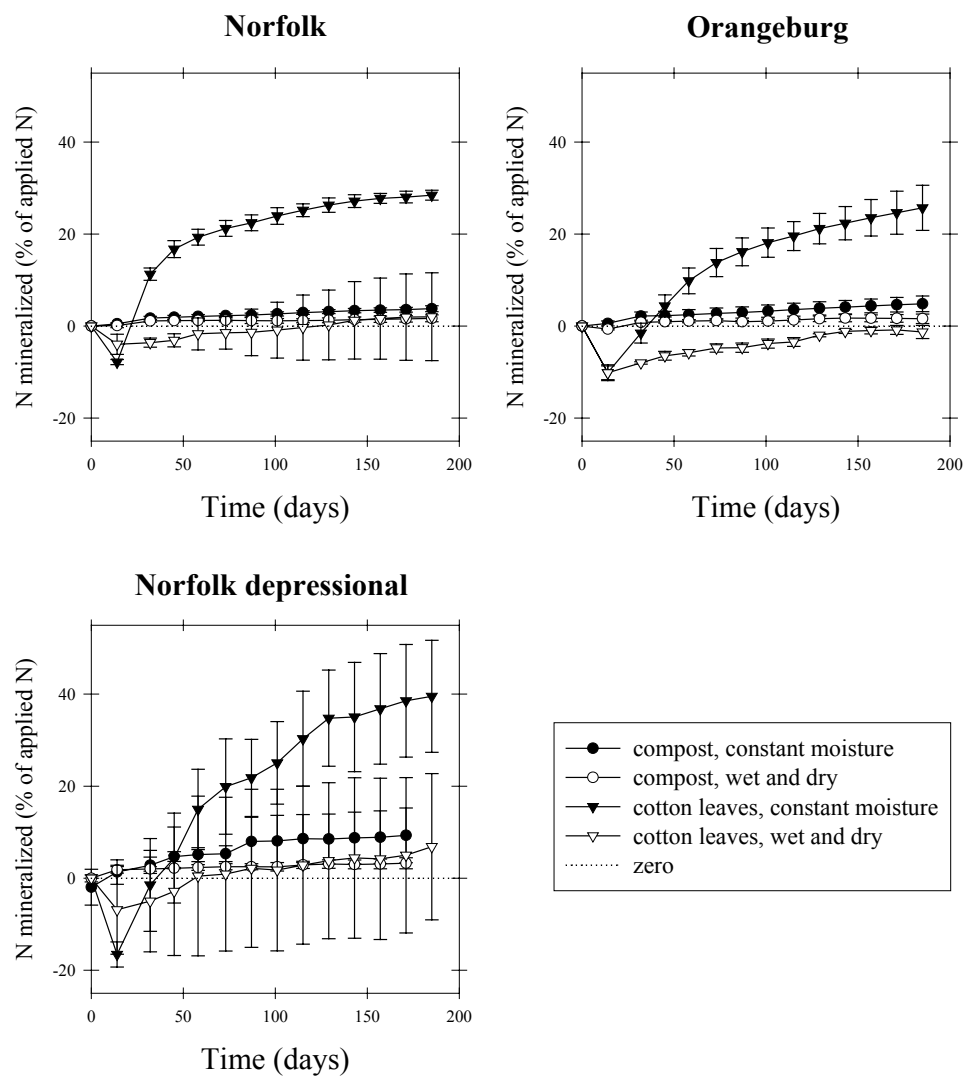


Figure 2.2 Nitrogen mineralized as % of N applied as cotton leaves or compost under different moisture regimes.

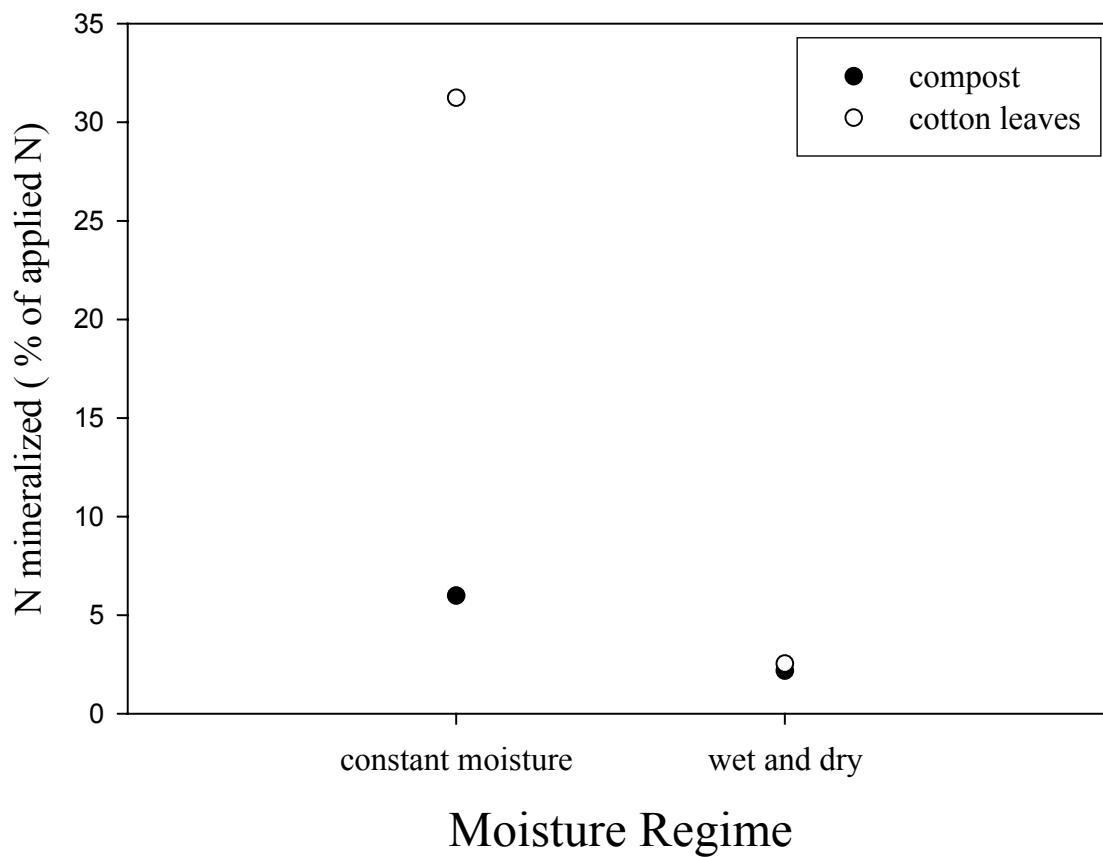


Figure 2.3 Plot of least squared means x moisture. The symbol is residue value. Wide gaps between values represent significant differences between treatment variables.

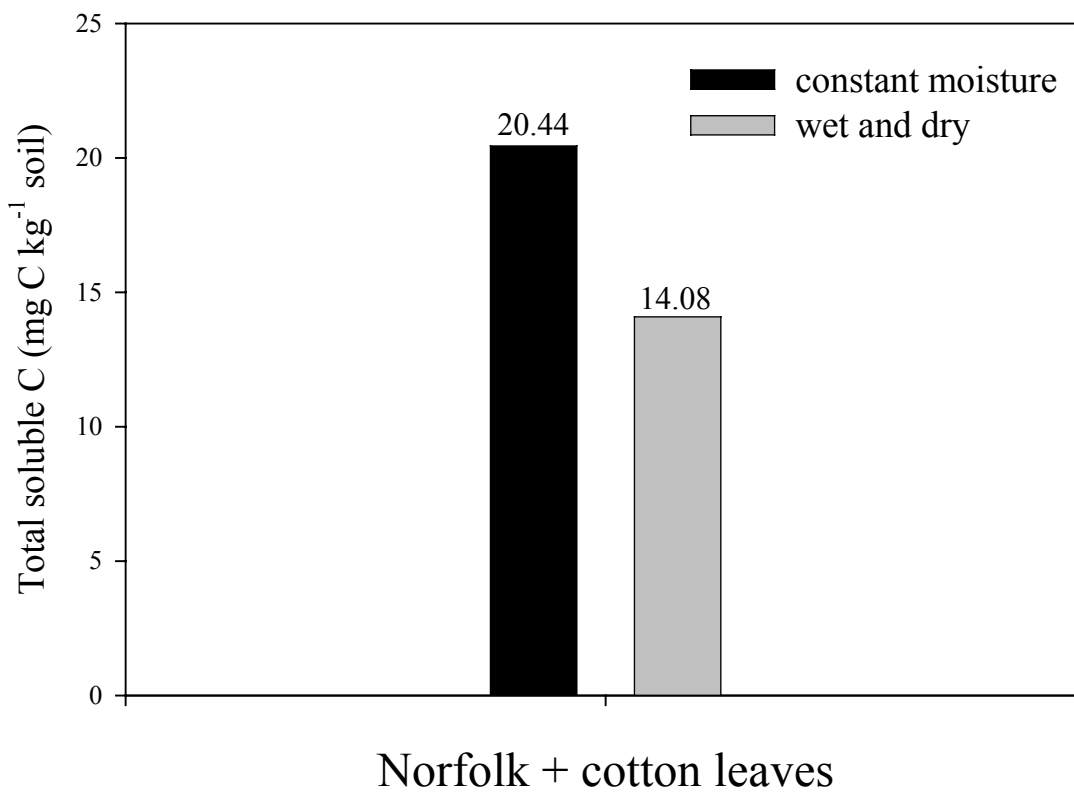


Figure 2.4 Total soluble C extracted from Norfolk soils with cotton leaves to evaluate whether treatments that underwent repeated wetting and drying experienced denitrification. Less total soluble C in the wet and dry treatment was an indication that denitrification had not occurred.



## **THE EFFECT OF WETTING AND DRYING ON CARBON MINERALIZATION**

### **Introduction**

Carbon mineralization rates in soils are dependent on a number of factors, including soil moisture, temperature, texture, microbial populations, and residue composition and placement. Researchers have studied the effect drying and rewetting has on C mineralization rates to determine whether it differs significantly from soils maintained at a constant moisture content. Soils in the field do not stay at a constant moisture level, but fluctuate according to environmental conditions. Birch (1958) found drying and rewetting released a flush of mineralized C, though the amount mineralized decreased after each successive rewetting episode. Wet-dry cycles also affected amounts of dissolved organic carbon measured in soils, according to Lundquist et al. (1999).

The purpose of this experiment was to determine the effect frequent drying and rewetting had on C mineralization rates of soil organic matter and cotton leaf or compost residues, in coastal plain soils of the southeastern United States.

### **Materials and Methods**

Three soil samples were collected from the upper 0.15 m of a field under conventional tillage in the coastal plain of Georgia to provide a range of clay and soil organic matter contents. The Norfolk loamy sand (coarse-loamy, kaolinitic, thermic Typic Kandiudults) contained both the lowest clay percentage and the lowest soil organic

matter. The Orangeburg sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiodults) contained the highest clay content and the medium level of soil organic matter of the selected soils. The Norfolk depressional sandy loam (fine-loamy, kaolinitic, thermic Arenic Kandiodults), a taxadjunct to the Norfolk series, contained a medium clay level and the highest soil organic matter content of the three soils (Table 2.1). Soils were air dried in the laboratory, crushed, then sieved through a 2-mm sieve to remove small rocks and non-decayed crop residue, which constituted less than 1% of the soil by weight. The water content of the soils at field capacity ( $-0.01$  MPa) was determined by using a pressure chamber. Three replicates of each soil were placed into steel cores with a 2-cm radius and 0.9-cm height and saturated with deionized water at atmospheric pressure. The samples were placed into the pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA) and brought to  $-0.01$  MPa, weighed, dried in an oven at  $105^{\circ}\text{C}$  for 48 hours, then weighed again (Klute, 1986). A subsample of each soil was analyzed for C and N with a Carlo-Erba NA 1500 Analyzer for Carbon and Nitrogen, Milan, Italy, according to the methods described by Kirsten (1983).

The study treatment design was a factorial experiment of three soils (Norfolk, Orangeburg, Norfolk Depressional), three residues (none, cotton leaves, compost) and two soil water levels (constant wet and alternating wet and dry). Organic residues chosen for the study were cotton leaves and compost. The compost selected began as a mixture of grass clippings, hardwood leaves, and pine needles. Windrows were stacked 120 cm high and mixed until the materials went through a peak heat. Both residues were oven-dried at  $105^{\circ}\text{C}$  for 96 hours. Samples were then sieved in a 2-mm sieve, composited, and ball milled. A subsample was analyzed for C and N (Table 2.2) with a Carlo-Erba NA

1500 Analyzer for Carbon and Nitrogen, Milan, Italy (Kirsten, 1983) by dry micro-Dumas combustion. Cotton leaves were applied at  $2057 \text{ mg kg}^{-1}$  soil, and compost was applied at  $15000 \text{ mg kg}^{-1}$  soil. After placement of a  $0.45\text{-}\mu\text{m}$  Supor® 450 membrane filter (Gelman Sciences, Ann Arbor, MI) filter in the bottom of a buchner funnel, residues were mixed with soils by placing the weighed soil and residue into the buchner funnel, then stirring with a glass rod for 5 min. Soil that fell through the bottom of the buchner funnel was captured by placing the funnel on paper during stirring, then pouring the soil back into the buchner funnel. There were three replicates of all treatments.

Treatments were divided into two systems. Those maintained at field capacity for the duration of the study were kept in an aquarium (25.5 x 50 x 30.5cm) whose external surface was taped with black duct tape to prevent algae growth. The bottom 2 cm of the tank held deionized water to help maintain high humidity. The top of the tank was covered by a stainless steel lid with 4-mm entry and exit ports. Air was pumped in by means of two Second Nature® Challenger 1™ aquarium air pumps providing  $4\text{L min}^{-1}$  air through Tygon® tubing into the bottom of a carboy partially filled with 4L deionized water. Moisture saturated air then exited through the top of the carboy via Tygon tubing into the entry port for the tank. Treatments that were dried and rewetted were stored in the high humidity tank for 3 d, then transferred to an identical tank that had laboratory air pumped into a dry glass bottle which then flowed into the entry port of the stainless steel lid. The bottom of the dry tank contained no water, but instead held 454-g Drierite® (W.A. Hammond Drierite Co., Xenia, OH) anhydrous calcium sulfate, that was changed daily. Soils were dried down in this tank for 11 d before being rewetted. Temperature and moisture levels inside the tanks were recorded for both treatments two times per

week. Each treatment was brought to field capacity with deionized water, then incubated either at a constant humidity of ~98% RH throughout the experiment, or incubated at ~98% RH for 3 d, then transferred to the dry tank for 11 d.

Carbon dioxide levels were determined daily for the first 48 hours after rewetting, then on an approximately every-other-day basis, by sealing the bottom of the porous Buchner funnel with Saran® Wrap, then recording the time the treatment was sealed. The seal was obtained by laying the pre-cut Saran square over the Buchner funnel bottom, then pressing the flanged Buchner funnel top firmly into the Buchner funnel bottom. The top was sealed by a flanged poly-vinyl chloride (PVC) disk with a rubber O-ring, pressed firmly into the Buchner funnel top. The PVC lid had a rubber septum fitted into the middle of the lid that allowed for CO<sub>2</sub> extraction by syringe. After waiting approximately one hour, the needle of a 2-mL syringe was inserted, plunger depressed, into the headspace of the treatment. The plunger was pulled to 2 mL, then depressed five times to circulate the air trapped in the soil pore space. Then 2 mL of headspace air was extracted and injected into a 2-mL glass vial with a septum lid. The CO<sub>2</sub> extraction time was recorded. Lids and Saran wrap were immediately removed from the treatments, following CO<sub>2</sub> extractions. All glass vials were sealed out-of-doors between 1900 and 2200 hours to ensure consistent background CO<sub>2</sub> levels, and after injection, stored at 4° C until analysis. The headspace for each treatment was determined by measuring the distance from the top of the soil treatment to the top of the Buchner funnel top, then subtracting the depth of the PVC lid flange. The diameter of each Buchner funnel was measured. Bulk densities ( $\rho_b$ ) of each treatment were measured and used to calculate porosity based on an assumed particle density ( $\rho_s$ ) of 2.65 g mL<sup>-1</sup>. The predominate

particles are quartz. Porosity was calculated as:  $1 - (\rho_b/\rho_s)$ . Relative air porosity was then calculated by subtracting the portion of total porosity occupied by liquid (1g = 1mL).

Because some soil was lost by leaching, we needed to know the amount of oven dry soil in the buchner funnel at each CO<sub>2</sub> measurement. Using the air dry weight (g) taken on day 210, and the oven dry weight taken on day 213 (at the end of the experiment when this could be measured), the oven dry weight for each day was calculated by:

$$\text{Weight of oven dry soil on day } Z_n = \text{weight of air dry soil on day } Z_n - (\text{air dry weight day } 210 - \text{oven dry weight day } 213) \quad [2.1]$$

Where  $Z_n$  represents the leaching day, and  $n$  varies from 1 to 13, e.g.  $Z_1 = \text{day } 14$ ,  $Z_2 = \text{day } 32$ , etcetera.

This relative air porosity was added to the head space above the soil to reach total head space (mL). CO<sub>2</sub> was analyzed on a Varian Star 3600CX (Varian Analytical Instruments, Sugar Land, Texas) gas chromatograph, which determined concentration based on thermal conductivity. CO<sub>2</sub>-C evolution rate was calculated by the equation:

$$\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil } \times \text{ hr} = \% \text{ CO}_2 \text{ in sample } \times (4.95702 \mu\text{g CO}_2\text{-C/mL}) \times \text{ mL headspace} \quad [2.2]$$

based on the equation to calculate a 1% CO<sub>2</sub> standard. Since there are 0.0413091 mol CO<sub>2</sub> per L CO<sub>2</sub> at 22° C, and  $12 \times 10^6 \mu\text{g CO}_2\text{-C per mol CO}_2$ , a 1% CO<sub>2</sub> standard contains 4.957092  $\mu\text{g CO}_2\text{-C per ml}$  according to the following equation:

$$(1\text{mL CO}_2/100\text{mL std}) \times (0.0413091 \text{ mol CO}_2^*/\text{L CO}_2) \times (1 \text{ L CO}_2/1000\text{mL CO}_2) \times (12 \times 10^6 \mu\text{g CO}_2\text{-C/mol CO}_2) = 4.957092 \mu\text{g CO}_2\text{-C/mL standard. [2.3]}$$

\* at 22° C.

## Results and Discussion

For all control soils (those without residue), the Orangeburg soil maintained at constant moisture mineralized the most C at 493.3 mg kg<sup>-1</sup> at 185 d. The Norfolk soil at constant moisture mineralized the least C at 257.4 mg kg<sup>-1</sup> at 185 d although it was statistically identical to the Norfolk subjected to repeated wetting and drying that mineralized 258.8 mg kg<sup>-1</sup> (Figure 3.1). Analyzed by moisture treatment, the Norfolk and Norfolk depressional mineralized similar amounts of C, but the Orangeburg treatment that was repeatedly dried and rewetted mineralized 57% of the treatment under constant moisture. By itself, moisture was not significant in determining mineralized C at the  $\alpha=0.05$  level (p value = 0.0636). Soil type, by itself, was significant at the  $\alpha=0.05$  level, (p value = 0.0088). There was a significant interaction between soil x moisture at the  $\alpha=0.05$  level (p value = 0.0034), therefore soil type and moisture were retained in the statistical model. Coefficient of variation rates averaged 42% with a range from 4% to 138% within replicates of treatments. The Orangeburg and Norfolk depressional tended to mineralize more C sooner than the Norfolk soil. By 32 d, the Orangeburg had mineralized between 41.8 and 46.5% of the total mineralized C at 185 d, depending on moisture treatment (Figure 3.2). Similarly, by 32 d, the Norfolk depressional had

mineralized between 39.0 and 46.3% of the total mineralized C. By contrast, the Norfolk mineralized from 24.9 to 27.7% of the total C mineralized, by 32 d. The Norfolk soil initially contained more C than the Orangeburg, but is a loamy sand, while the Orangeburg is a sandy loam. The Norfolk tended to dry more quickly than the Orangeburg, as was noted by visual observation during the experiment, and therefore spent a larger percentage of the time during the experiment in a “dry” state. A preliminary study conducted as described in Chapter 1 revealed the Norfolk lost water content (from field capacity to air-dry) in 85% of the time as the Orangeburg (Figure 1.1). This may help explain reduced mineralization for the treatment undergoing repeated wetting and drying, but does not explain the treatment maintained at constant moisture. In fact, previous researchers found clay particles provided a “protective effect” for microorganisms, resulting in lower rates of N mineralization for soils with higher clay contents ( Birch 1958; Sorensen 1981; van Veen et al. 1985; Ladd et al. 1992), but that the influence of texture may be less important for C mineralization (Hassink et al. 1993; Hassink et al. 1994). In a study on N mineralization, Strong et al. (1999) observed that when soils were treated with clover-derived substrate, clay increased N mineralization and nitrification rates. They state that this may have been because clay limited the diffusion of partially decomposed organics (i.e. C-based compounds) away from the decomposing microbial population, thereby helping to facilitate more complete decomposition of the organic material. If C-based compounds are more limited in diffusion away from the decomposing microbial population, one would expect higher rates of CO<sub>2</sub> production as C is more completely mineralized.

For all soils treated with cotton leaves, the Norfolk depressional under constant moisture and under repeated wetting and drying, as well as the Orangeburg soil under constant moisture released the most CO<sub>2</sub> (681, 685, and 674 mg kg<sup>-1</sup>, respectively). The Norfolk soil with cotton leaves under repeated wetting and drying released the least CO<sub>2</sub> over the study (437 mg kg<sup>-1</sup>) but was similar to the Orangeburg under wetting and drying at 467 mg kg<sup>-1</sup>. Although the initial application rate of C from cotton leaves was 24.7% of the application rate for C from compost (Table 2.1), in all cases treatments with cotton leaves mineralized more C than treatments with compost for a given soil (Figure 3.1).

The Orangeburg with cotton leaves under constant moisture mineralized 41.8% of total C mineralized, after 32 d. The Orangeburg with cotton leaves under wetting and drying mineralized 59.9% after 32 d. The Norfolk soil with cotton leaves under constant moisture mineralized 52.2% after 32 d, and the Norfolk soil with cotton leaves under wetting and drying mineralized 57.7% after 32 d. The Norfolk depressional with cotton leaves under constant moisture mineralized 53.5% after 32 d, and the Norfolk depressional with cotton leaves under wetting and drying mineralized 52.7% after 32 d. Across soils and moisture treatments, roughly half the C mineralized as CO<sub>2</sub> after 185 d had mineralized after the first month.

For all soils with compost, the Orangeburg under constant moisture mineralized the most C as CO<sub>2</sub>, at a mean value of 592-mg kg<sup>-1</sup>. The Orangeburg under wetting and drying, on average, mineralized the least C at 330-mg kg<sup>-1</sup>. By contrast the Norfolk and Norfolk depressional soils under different moisture treatments mineralized similar amounts of C (Figure 3.1).



When mineralized C is evaluated in terms of percent C applied, certain distinct patterns emerge (Figure 3.3). Means and standard deviations were obtained by subtracting the mean value of the control soil mineralized C from each replication of the soil plus residue mineralized C value, then dividing the differences by the amount of initial C applied, and multiplying by 100. In a few instances in the Orangeburg soil, the treatments with residue evolved less CO<sub>2</sub> than the mean value of CO<sub>2</sub> evolved from the control soil, resulting in a negative slope (Figure 3.3).

Soil type was significant at the  $\alpha = 0.05$  level, as was type of residue. Statistical analysis using Tukey's method revealed the Norfolk depressional and Orangeburg were significantly different (Table 2.3). Moisture was not significant, nor were any interactions between variables.

Residue type played an important role in the amount of C mineralized. Cotton leaves mineralized between 20.3% and 37.9% (mean values) of their C content after 185 d, depending on the soil type and moisture treatment (Figure 3.3). Compost, by contrast, mineralized between 0.7% and 6.15% of their C content (mean values) after 185 d (Figure 3.3). In cotton leaves, C underwent rapid mineralization, with over half the total C mineralized by 32 d, with the exception of the Orangeburg soil under constant moisture (Figure 3.3). In the case of the Norfolk soil under repeated wetting and drying, 93% of the C mineralized had mineralized after 32 d. This rapid mineralization is supported by observed N immobilization in soils with cotton leaf residues during the first 14 to 32 d as shown in Chapter 2.

Compost showed very little C mineralization. The material underwent thorough decay prior to use in the experiment, leaving humic materials that were apparently more resistant to decomposition. The C mineralization data are consistent with the N data for soil with compost in Chapter 2, which also showed very little mineralization (Figure 2.5).

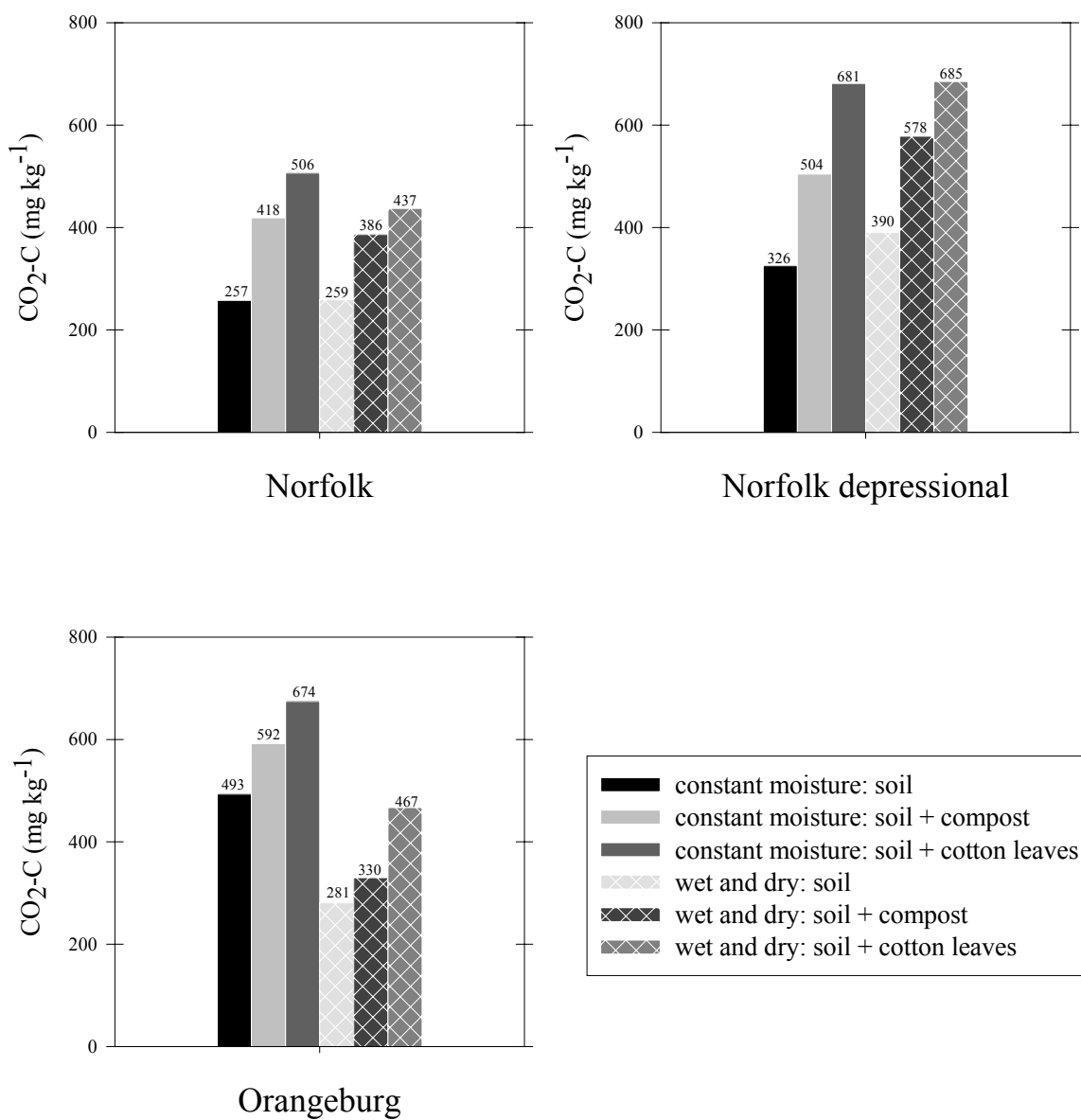


Figure 3.1 Total mean integrated CO<sub>2</sub>-C after 185 d from the three selected soils at various combinations of moisture regimes and residue types.

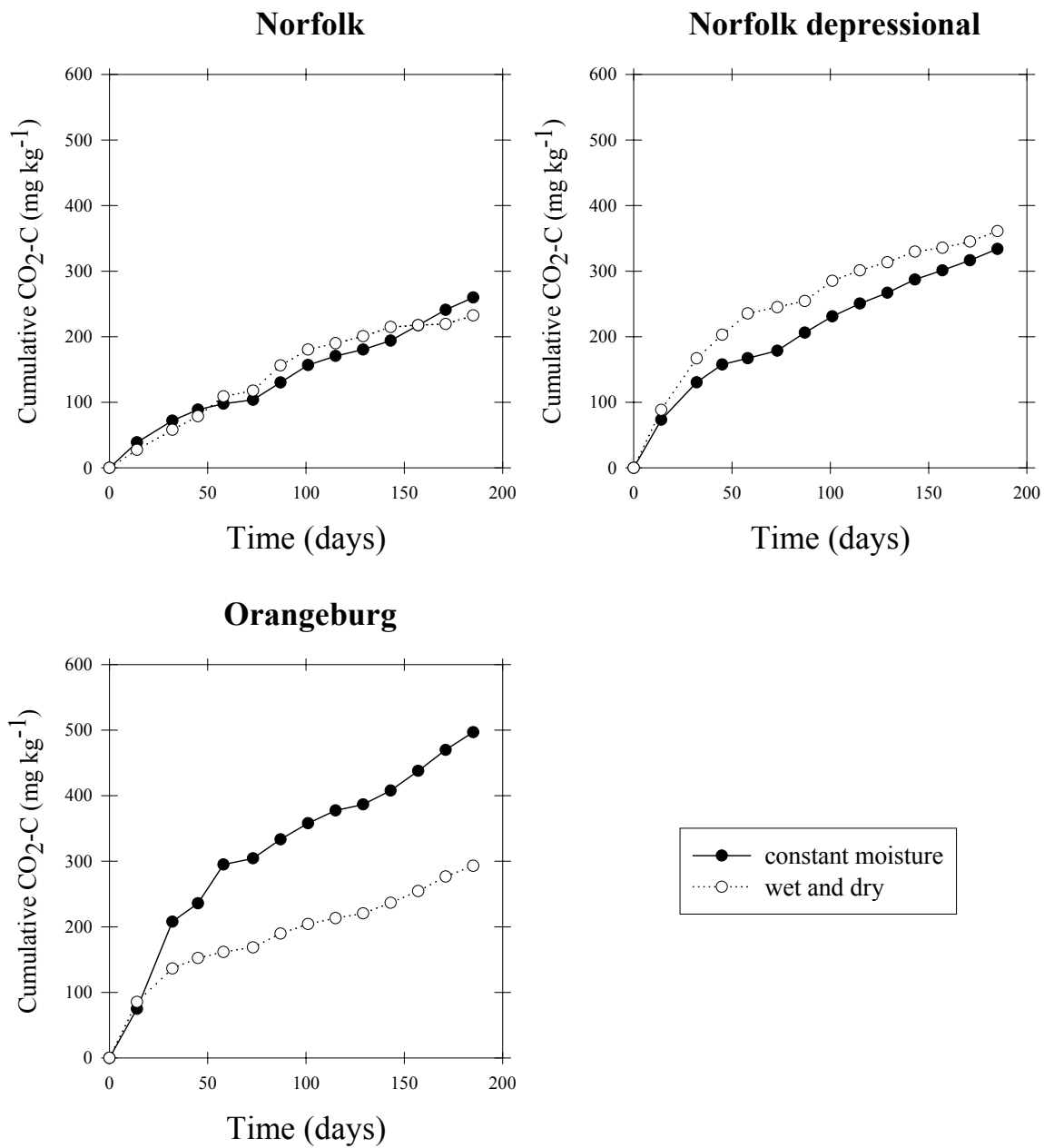


Figure 3.2 Mean cumulative CO<sub>2</sub>-C in the three control soils with no residue applied, by moisture regime.

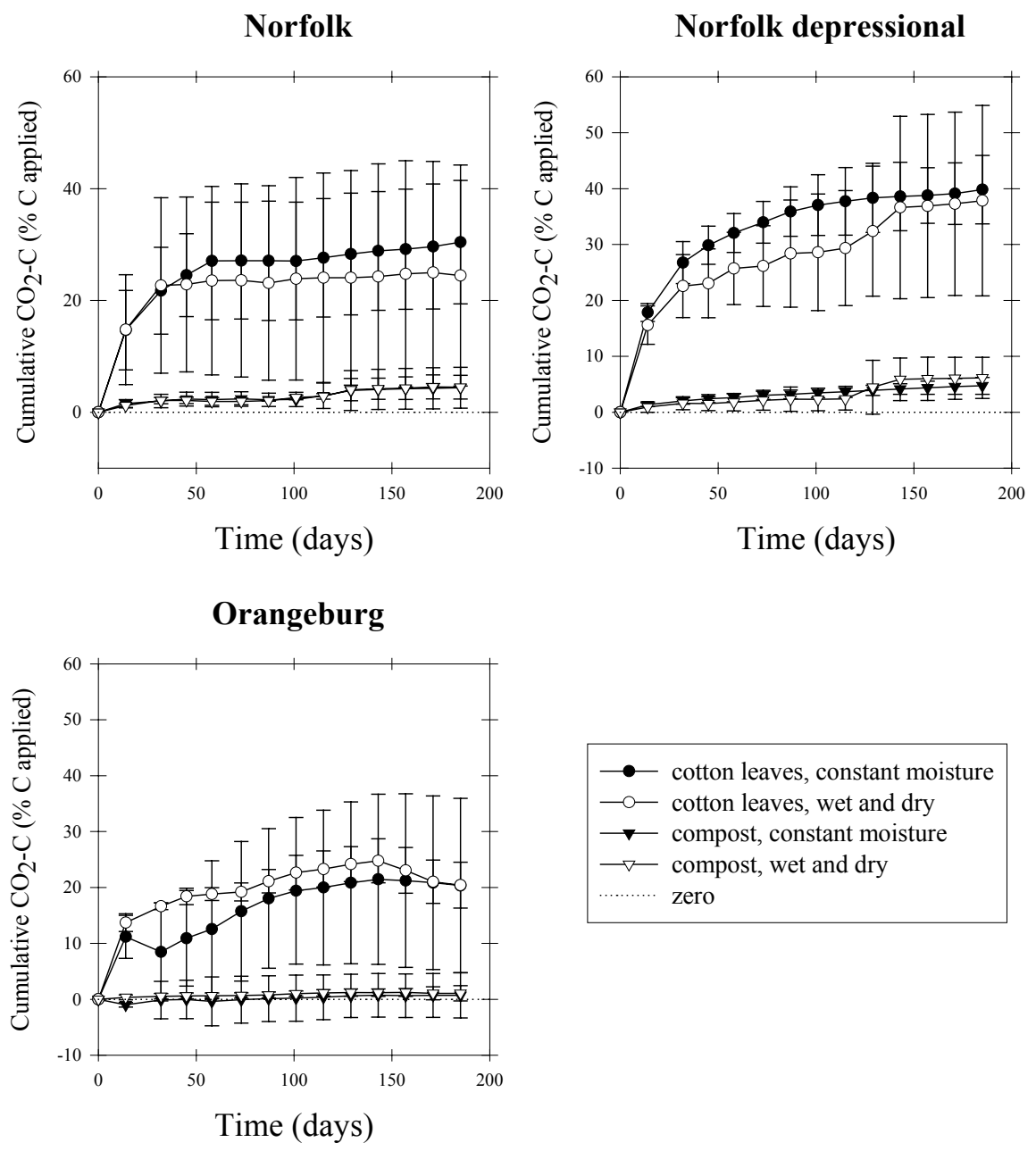


Figure 3.3 Cumulative CO<sub>2</sub>-C, evolved from cotton leaves or compost, by moisture regime.

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